New Series Vol. XVI No. 63 July 1952

Price 20s. net

ANNALS OF BOTANY

EDITED BY

W. H. PEARSALL

D.Sc., F.R.S.

Quain Professor of Botany
University College, London

ASSISTED BY

D. G. CATCHESIDE, D.Sc., F.R.S.

Professor of Genetics
University of Adelaide, South Australia

F. G. GREGORY, D.Sc., F.R.S.

Professor of Plant Physiology
Imperial College of Science and Technology, London

T. M. HARRIS, M.A., PH.D., F.R.S.

Professor of Botany
The University, Reading

OXFORD : AT THE CLARENDON PRESS

LONDON : GEOFFREY CUMBERLEGE

PRINTED IN GREAT BRITAIN
BY CHARLES BATEY AT THE UNIVERSITY PRESS, OXFORD

1952

CONTENTS

NOTICE TO CONTRIBUTORS

Contributors should address their papers to Prof. W. H. PEARSALL, Department of Botany, University College, London, W.C. 1.

Papers sent for publication *should be type-written. They should include a short abstract and should conclude with a summary of the contents.* In view of the increasing numbers of manuscripts submitted, the Editors desire to impress upon contributors the importance of conciseness. Bodies of quantitative data, too extensive for complete publication, should be summarized for discussion and the originals deposited in the Archives established for this purpose in the British Museum (Natural History), South Kensington, S.W. 7. The Editors suggest that the longer papers should not exceed 12,000 words. Contributors will receive one proof in page. An allowance at the rate of ten shillings per sheet of sixteen pages is made for alterations in the proof (printers' errors excepted), and contributors will be responsible for any excess.

ILLUSTRATIONS. These where possible should be suitable for reproduction as line-blocks in the text. Where lithographic or collotype plates are required the figures should be planned so as to fill properly a 4to or an 8vo plate. The maximum space available for figures in a 4to plate is $8\frac{1}{4} \times 11\frac{1}{4}$ inches, in an 8vo plate $8\frac{1}{4} \times 5\frac{1}{4}$ inches. The lettering of figures, whether text-figures or those of plates, should be in pencil.

The Journal is published four times yearly by Geoffrey Cumberlege, Oxford University Press, Amen House, Warwick Square, London, E.C. 4, to whom subscriptions and all communications, other than editorial, should be addressed.

The subscription price for four numbers is 50*s.* (foreign postage, 1*s.* 6*d.* extra), single copy 20*s.* net. Cloth cases may be obtained, price 7*s.* 6*d.*

FIRST SERIES (VOLUMES 1–50)

Orders and inquiries, other than those from U.S.A., for copies of the first series (Volumes 1–50) of the Annals of Botany should be addressed to The Assistant Treasurer, Annals of Botany Company, Botany School, Cambridge, England. Messrs. J. S. Canner & Co., 46 Millmont Street, Boston 19, Mass., are the sole distributors in the U.S.A. and all American inquiries should be addressed to them.

Stocks of Volumes 1, 2, 19–25, 28, 30–34, 36–40 *are exhausted.* Most of the early volumes are in sheet form, unbound; and in a few cases the volumes are slightly imperfect.

The prices of the back numbers, including packing and postage, are £2. 10*s.* 0*d.* a volume, whether in the form of bound parts or separate sheets (half price if imperfect); 20*s.* a single part (bound); 10*s.* a single part (in sheets). Indexes at usual published prices.

Cheques, other than those from U.S.A., should be made payable to The Annals of Botany Company and sent to The Assistant Treasurer.

The publishers are signatories to the Fair Copying Declaration in respect of this journal. Details of the Declaration may be obtained from the office of the Royal Society upon application.

The Ascogenous Hyphae of *Pyronema confluens*[1]

BY

IRENE M. WILSON

(*University College of Wales, Aberystwyth*)

With Plates XVIII and XIX and nineteen Figures in the Text

Abstract

The ascogenous hyphae arise from the oogonium, opposite groups of nuclei, as minute, enucleate papillae. Nuclei pass into them singly, rarely two at a time, and a knob-like swelling is formed, containing several nuclei and later growing out into one or more branches. The nuclei are in single file in the branches and irregularly arranged in the bulbous base. There are frequently two nuclei in a leading position at the tip of the young branch, but the nuclei may become more evenly spaced as the hypha elongates. The nuclei undergo a simultaneous mitosis. The spindles of the dividing nuclei in the branches are not parallel and this is, therefore, not a conjugate division. Walls are formed as ingrowing rings across the spindles so that the ascogenous hypha, when septate, has a uninucleate end cell followed by one, or usually more, binucleate cells and a basal bulb containing a variable number of nuclei.

Croziers are formed as lateral, hooked outgrowths from the binucleate cells. After a simultaneous mitosis of the two nuclei a uninucleate end cell, a binucleate penultimate cell, and a uninucleate stalk cell are formed. Thus, the division in the crozier and that in the ascogenous hypha are alike.

The binucleate cell of the crozier may proliferate to form another crozier, or it may form an ascus after the fusion of its two nuclei. The stalk and terminal cell of the crozier may anastomose and grow out to form a lateral crozier.

The chromosome number in the mitosis in the ascogenous hypha is twelve and there are twelve bivalents at the first division of meiosis in the ascus.

The effect of increasing the illumination of the cultures with an electric lamp in addition to diffuse daylight is to ensure the further development of all early formed sexual organs, to make the ascogenous hyphae develop rapidly, to make the latter short and curved in form with few binucleate cells, and to increase the tendency towards a period of erect proliferation before the formation of the asci and lateral proliferation begin.

The bearing of the results on current theories of sexuality in the Ascomycetes is discussed.

Introduction

A STRIKING feature of the literature on the cytology of the Ascomycetes is the lack of information on the behaviour of the nuclei in the ascogenous hyphae. Most authors have been more concerned with the sexual organs or with the asci than with the phase connecting these two more conspicuous stages in the life-cycle. *Pyronema confluens* is one of the few species

[1] *P. confluens* Pers. ex Tul. = *P. omphalodes* (Bull. ex Fr.) Fuckel.

[Annals of Botany, N.S. Vol. XVI, No. 63, July, 1952.]

in which the development of the ascogenous hyphae has been described fully, but unfortunately the accounts which have been given are as varied as their authors' views on sexuality in this plant.

It is well known that the nature and origin of the nuclei entering the ascogenous hyphae from the oogonium have been the subject of much controversy. Views differ as to whether they are diploid nuclei resulting from a sexual fusion (Harper, 1900; Gwynne-Vaughan and Williamson, 1931; Colson, 1942), whether they are paired male and female nuclei whose association is maintained by conjugate mitoses (Claussen, 1907 and 1912), or whether they are entirely of ascogonial[1] origin (Dangeard, 1907; Brown, 1915, for var. *inigneum*; Moreau and Moreau, 1930; Raymond, 1934). This problem is considered only indirectly here.

Conflicting accounts have also been published of the development of the ascogenous hypha itself. The main points still at issue are the presence or absence of paired nuclei in the aseptate ascogenous hypha, the number of nuclear divisions which take place before septation, whether the nuclei divide conjugately with parallel spindles or simultaneously in single file and, hence, the origin of the two nuclei in the binucleate cells after septation and the position of the asci, terminal or lateral, on the ascogenous hypha. It is this second group of problems which this investigation attempts to solve, but the results obtained may throw some light on the problem of sexuality also.

Material and Methods

The original material used in this investigation consisted of a few ascocarps growing on sterilized soil at the Welsh Plant Breeding Station on June 7, 1938.

The earlier workers on *Pyronema* produced successive crops of fruits by watering, at intervals, the burnt soil on which the fungus naturally occurred. Dangeard (1907) cultivated it on agar to which powdered charcoal on which the fungus had been growing was added, and Moreau and Moreau (1930) used a similar medium with the addition of 1 part per 100 of glucose. The modification of Coon's agar recommended by Robinson (1926) and the maltose-agar of Raymond (1934) did not prove to be satisfactory in this investigation, but Claussen's agar (Claussen, 1912), also used by Gwynne-Vaughan and Williamson (1931) and by Kerl (1937), gave excellent results. All the figures in this paper are from material grown on this medium. Cultures were also sometimes grown on sterilized soil for comparison with those on artificial media.

It has been shown that in addition to a satisfactory nutrient medium, suitable conditions of humidity, temperature, and light are necessary for the development of ripe fruit-bodies. The scattered observations of earlier

[1] Dangeard considered that the sexual fusion occurred in the young ascus and called the oogonium and antheridium of other authors the ascogonium and the trophogone respectively. Harper used the term 'ascogonium' for the oogonium after fertilization. The term 'ascogonium' was first suggested by Kihlman (1885) for the female sexual organ since it gave rise to ascogenous hyphae and asci.

authors on this subject are summarized by Robinson (1926), who added that the light stimulus, which is necessary for the formation of the characteristic pink pigment and of the sexual organs, becomes effective after a mechanical check to mycelial growth has occurred. In artificial cultures this check is brought about by the mycelium reaching the edge of the Petri dish. Kerl (1937) has, in the main, confirmed Robinson's results and has greatly extended them by experimental work on the effect of light of different intensity and duration on the rate of development and on the effect of growth substances.

The conditions cited as necessary for development by these authors can be satisfied by the following simple technique. Cultures were grown on Claussen's agar (generally from a large inoculum containing an ascocarp as well as some mycelium) and were kept in an incubator at 22° C. for 48 hours. By this time the mycelium had reached the edge of the Petri dish. The dishes were then exposed to light at room temperature. Although fruit-bodies are formed in abundance with 12 hours' exposure to diffuse daylight daily, there are always a few rosettes of sexual organs which fail to develop into ripe ascocarps under these conditions (Text-fig. 8). Instead, they grow out into vegetative hyphae and form dark brown sclerotia with oily contents. For this reason additional illumination from a 100-watt lamp for varying periods and at varying distances was always used to supplement daylight, except when growth under limiting conditions of light was being specifically studied. The additional warmth supplied by the lamp, if placed near the culture, was advantageous to growth as long as the temperature was not allowed to rise above 30° C. Sclerotia also tend to develop in unsuitable conditions of temperature and humidity. They are also formed from the last of the successive crops of sexual organs which develop on the culture, and for this reason the story of the normal course of development must be built up from the most advanced stages present.

All stages were examined in uncut material as a check to the microtomed sections. The development of the ascogenous hyphae can be followed by mounting fresh material in water and pressing on the cover-slip until the oogonia with their crown of ascogenous hyphae are separated out. In order to see the nuclei at these stages, techniques, similar to those used for smears of pollen grains and root-tips, can be applied. After fixation in acetic alcohol, material was stained with orcein, or with aceto-carmine (Godward, 1948). Gentian violet or Heidenhain's haematoxylin was also used successfully for smears. Germinating spores were similarly stained.

Material for microtomed sections was fixed either in Merkel's fluid or in Flemming's fluid (half strength). After embedding in paraffin wax, sections were cut from 6 to 16 μ, but mainly between 8 and 12 μ. The most satisfactory stain for the detail of nuclear structure and for chromosome counts was gentian violet, but Heidenhain's haematoxylin also proved very useful, especially for studying the distribution of resting nuclei.

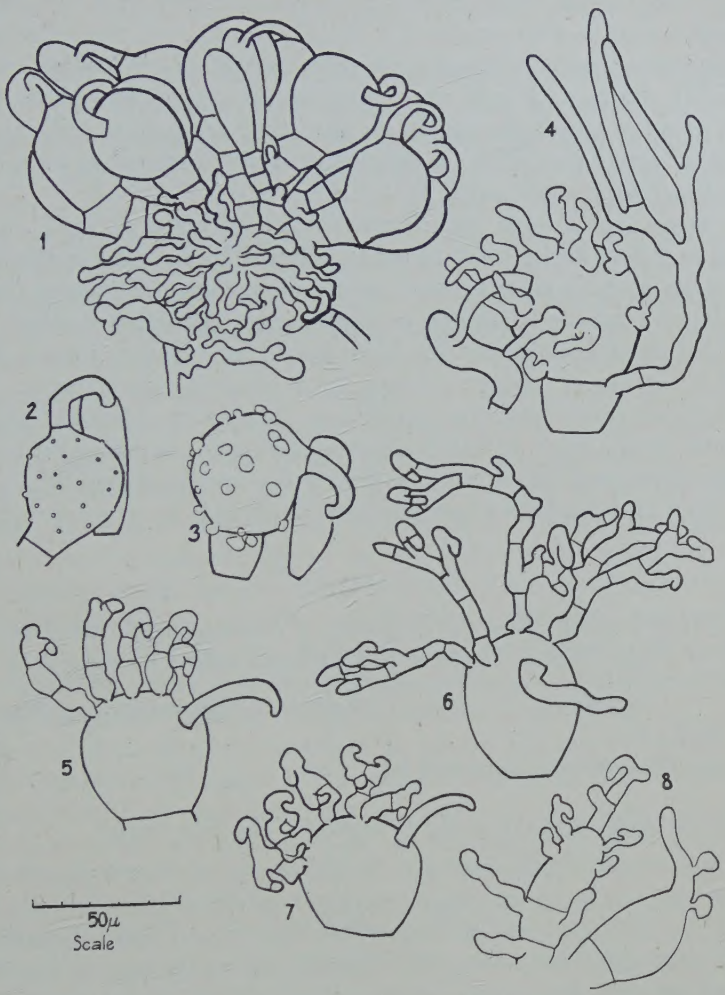
Development of the Ascogenous Hyphae

1. *The origin of the ascogenous hyphae*

At the time of origin of the ascogenous hyphae the antheridium is nearly emptied of its contents but contains some darkly staining peripheral cytoplasm (Pl. XVIII, Figs. 1, 5). The antheridium is, and remains, in open communication with the trichogyne. The trichogyne nuclei have degenerated, but sometimes a few recognizable nuclei, probably antheridial, can be seen at its distal end (Pl. XVIII, Fig. 1). The direction of the convex striations in the deeply staining material of the trichogyne suggests the movement of a thick, viscous substance towards the oogonium (Pl. XVIII, Fig. 1). There is a wall between the oogonium and the trichogyne, its small central perforation being usually blocked by a small, deeply staining granule or pore-plug as Buller (1933) calls it (Pl. XVIII, Fig. 1). On a few occasions this granule has been seen displaced and deeply staining material observed passing into the oogonium from the trichogyne through the perforation (Pl. XVIII, Fig. 11). The wall between the oogonium and the stalk cell below has a similar perforation which is also usually blocked by a pore-plug on each side (Pl. XVIII, Fig. 10). Thus the oogonium is intimately connected with the antheridium through the trichogyne, with the stalk cells bearing the sexual organs and the mycelium on which they are developed, and with the whole system of downwardly growing hyphae and upwardly growing aerial paraphyses which develop from the stalk cells (Text-fig. 1). There is some experimental evidence that the developing ascogenous hyphae derive food materials from these sources (Kerl, 1937).

Numerous ascogenous hyphae arise from the surface of each oogonium of the rosette of sexual organs (Text-figs. 1, 2, 3; Pl. XVIII, Fig. 2). They appear as minute, thin-walled, conical papillae and are, at first, without nuclei (Pl. XVIII, Fig. 2). At this time the nuclei in the oogonium are distributed in the peripheral cytoplasm. The arrangement of the nuclei can be seen in sections of the oogonium cut near the surface (Pl. XVIII, Figs. 1, 5) and in a median section of the oogonium (Pl. XVIII, Fig. 2). No regularly paired arrangement of the nuclei can be detected, nor are the nuclei spaced equidistantly. They lie singly, in pairs, or in small groups. The ascogenous hyphae appear to arise near to small groups of two or more nuclei, rather than in the spaces between them, and this may account for the fact, illustrated in Text-figs. 2 and 3, that the ascogenous hyphae are unevenly distributed over the surface of the oogonium. Just before the ascogenous hyphae are actually visible, gentle pressure on the living oogonium will make them spring out as minute papillae studding the surface of the wall. This suggests that the oogonial wall is thinner and less rigid at these points which are opposite groups of nuclei. The thin wall of the young ascogenous hyphae can be seen also in microtomed sections (Pl. XVIII, Figs. 2 to 8).

As each papilla becomes larger and more globose, the nuclei start to pass into it from the oogonium, one at a time, becoming slightly constricted and dumb-bell-shaped as they pass through the narrow neck separating the



TEXT-FIGS. 1–8. Development of ascogenous hyphae up to formation of croziers. (All drawn from uncut material.) Fig. 1. Rosette of sexual organs. Oogonia and antheridia as dichotomies of separate branches. Trichogynes of some oogonia attached to antheridia. Anchoring hyphae and paraphyses arising from stalk cells of sexual organs. Fig. 2. Oogonium with trichogyne attached to antheridium. Ascogenous hyphae arising from oogonium as minute, irregularly spaced, papillae. Fig. 3. 'Knob' stage of ascogenous hyphae. Fig. 4. 'Knobs' growing out into branches. Paraphyses from stalk cell. Fig. 5. Short, septate ascogenous hyphae, curved at tip, characteristic of bright illumination. Fig. 6. Long, septate ascogenous hyphae formed in diffuse daylight. Small end cell not developing further. Croziers formed laterally from (binucleate) cells below end cell. Fig. 7. Similar stage to Fig. 6, but grown in strong illumination. Fig. 8. Antheridium (on left) and oogonium (on right) forming vegetative hyphae in diffuse daylight.

enlarging bulb of the ascogenous hypha from the oogonium (Pl. XVIII, Figs. 3, 4, 5). Very occasionally two nuclei make their way in at the same time, from opposite sides of the papilla (Pl. XVIII, Fig. 6), and, when this happens, the ascogenous hypha becomes wide open, instead of constricted, at its base (Pl. XVIII, Fig. 7). Nuclei may continue to crowd into these rounded 'knobs' growing out of the oogonium until they are packed with nuclei, or, under other external conditions, development may proceed as soon as about four or five have passed in (Pl. XVIII, Figs. 8, 9).

In cultures which have been incubated for 48 hours at 22° C. and then exposed to diffuse daylight for 12 hours followed by 12 hours in the dark, the 'knob' stage is nearly always present as the most advanced stage at the end of the 24 hours after the beginning of illumination. Cultures similarly treated except that extra illumination has been given, for example from a 100-watt lamp at a distance of 6 inches, always show more advanced stages. Table I shows a typical result from one experiment. The more advanced condition of the culture, at this stage, with extra illumination is due to the earlier formation of sexual organs, and this in turn is influenced by the increased light intensity (Kerl, 1937) and the higher temperature (Raymond, 1934) produced by the lamp.

TABLE I

Average Time in Hours from the Beginning of Illumination for the Development of Three Critical Stages in P. Confluens *with a 12-hour Day*

Treatment.	'Knobs.'	First crozier.	Ripe asci.
Diffuse daylight	24	42	90
Diffuse daylight + 100-watt lamp at 6 in.	19	24	54

2. *From the 'knob' stage up to the formation of the first crozier*

The ascogenous hypha from the 'knob' up to the formation of the first crozier is strikingly influenced by external conditions both in its rate of development and in its external form. It is clear from Table I that with additional illumination from a 100-watt lamp at 6 inches the rate of development is much increased, and a comparison of Text-figs. 6 and 7 shows that the ascogenous hyphae formed are short and curved at the tip instead of long and straight.

From Table I it can also be seen that neither of the treatments listed gives a convenient time schedule for studying the septation of the ascogenous hypha, for in both this stage takes place at night. In practice, therefore, it is advisable either to cut down the duration of the extra illumination or to increase the distance of the lamp on the first day after removal of the cultures from the incubator (to ensure that the 'knob' stage is just beginning to grow out at the end of 24 hours) and then to give strong additional illumination on the second day. Under these conditions the 'knobs' will grow out into branches and become septate within about 3 hours on the second day of illumination.

The effect of the illumination is not direct, for the acceleration of growth

and the short, curved form of the ascogenous hypha may occur in the dark provided that the illumination has been sufficiently bright and prolonged earlier.

When cultures have been given extra illumination in addition to diffuse daylight, the 'knob'-like ascogenous hyphae may grow out into branches somewhat prematurely, that is, before many nuclei have passed into them. In bright light usually only one or two branches are formed from each 'knob' (Text-figs. 4, 7). The branch, at an early stage, often shows two nuclei in a leading position at the tip (Pl. XVIII, Fig. 9). The leading pair of nuclei may be followed by another pair, but the arrangement of the nuclei is less orderly at the base (Pl. XVIII, Fig. 10). The tip of the hypha is generally curved in bright illumination (Pl. XVIII, Figs. 10, 13, 15, 16). The nuclei are always in a single row, except at the base. They may become rather more evenly spaced out as the hypha elongates, but in bright light the rapid onset of mitosis coincides with a checking of this process. All the nuclei in the ascogenous hyphae, and also those remaining in the oogonium which bears them, divide simultaneously (Pl. XVIII, Fig. 13). This is the first mitosis to occur since the ascogenous hyphae began to form. The nuclei in the narrow branches of the ascogenous hyphae remain in single file during the division and the spindles do not slip past each other and are not parallel in the telophase (Pl. XVIII, Figs. 13, 14, 15, 16). This mitosis is, therefore, not a conjugate mitosis in the sense of Claussen (1912). In the lower, wider part of the ascogenous hypha the nuclei are irregularly arranged and the spindles lie in various planes and may sometimes be parallel (Pl. XVIII, Figs. 13, 14, 15).

At the end of the division, daughter nuclei are organized and the position of the former spindle is marked by a vacuole. A septum is formed as an ingrowing ring across the position formerly occupied by the spindle (Pl. XVIII, Fig. 16). In this way a uninucleate end cell, followed by a series of binucleate cells, is formed. The binucleate cells contain non-sister nuclei. Septa are not formed in the broad, basal bulb of the ascogenous hypha which contains a variable number of nuclei (Pl. XIX, Fig. 18). The small end cell is also clearly visible in fresh material of this stage (Text-figs. 5, 6, 7). The nucleus of the end cell remains small and this cell does not usually play any part in further development (Pl. XIX, Figs. 18, 20, 22, 23, and Text-figs. 6, 7).

The simultaneous mitosis of the nuclei followed by the septation of the ascogenous hypha stops the further migration of nuclei out of the oogonium. The oogonium may sometimes appear crowded with nuclei in the peripheral cytoplasm. This is due partly to the fact that only some of its nuclei had time to pass into the ascogenous hyphae, and partly to the fact that those remaining in it have divided.

The number of chromosomes present on the metaphase plate during the simultaneous division of nuclei in the ascogenous hyphae is twelve (Pl. XVIII, Fig. 13, top nucleus on right). Most of the other nuclei in this figure are cut, as might be expected in a microtomed section of a twisting hypha.

Exceptionally, in very bright light, the ascogenous hypha may be so short

and so hooked as to appear like a crozier (Pl. XIX, Figs. 22, 25, and Text-fig. 5), but such a short branch usually differs from a true crozier in having a multinucleate basal cell. The single, binucleate, penultimate cell does not form an ascus, but always forms a crozier (Pl. XIX, Fig. 22, on left). The uninucleate end cell does not usually play any further part in development, and Pl. XIX, Fig. 25, represents the only example seen in which the uninucleate end cell fused with the basal cell as so often happens in a crozier. The hypha on the right of Pl. XIX, Fig. 22, looks as if it might have done the same. Such an exceptionally short ascogenous hypha, resembling a crozier in some respects, is to be regarded as an extreme and limiting case of the normal development in bright illumination.

In diffuse daylight, with no extra illumination, or very little, the whole development takes place more slowly. More nuclei pass out of the oogonium, until it is left nearly empty, and crowd into the 'knobs'. This stage may occupy from 3 to 6 hours. Frequently two or three branches grow out from each 'knob' (Text-fig. 6). They take several hours to attain their full length and are, on the whole, longer and more straggling than those formed in bright light. They are not hooked at the tip (Pl. XVIII, Figs. 12, 14, 17, and Text-fig. 6). During the elongation of the ascogenous hypha the nuclei become widely spaced (Pl. XVIII, Fig. 12). They are in single file in the upper narrow branch and more irregularly arranged below. The simultaneous mitosis of the nuclei in the ascogenous hypha takes place as before (Pl. XVIII, Fig. 14) and results again in the formation of a uninucleate end cell followed by a series of binucleate cells (Pl. XVIII, Fig. 17) and a multinucleate basal cell.

Croziers are formed only from the binucleate cells of the septate ascogenous hyphae. The nuclei in these cells grow large and have well-marked nucleoli and contrast sharply with the nucleus in the end cell and with the nuclei in the broad basal bulb which will not take any further part in development (Pl. XIX, Fig. 18). A lateral outgrowth is formed from the binucleate cell into which the two nuclei pass (Pl. XIX, Fig. 18); this becomes curved, the two nuclei undergo a simultaneous mitosis (Pl. XIX, Figs. 19, 20), and a wall is formed across each spindle region as an ingrowing ring (Pl. XIX, Fig. 19) cutting out a uninucleate end cell, a binucleate penultimate cell at the apex of the hook containing non-sister nuclei, and a uninucleate stalk cell (Pl. XIX, Figs. 20, 23). The chromosome number at this mitosis is again twelve. The decrease in size as the daughter nuclei are formed should be noted (Pl. XIX, Figs. 19, 20, 24).

The uninucleate end cell of the original ascogenous hypha does not form a crozier. All croziers are lateral. Owing to the fact that, in bright light, the tip of the ascogenous hypha may be strongly curved, the morphologically lateral crozier may appear to be terminal (Pl. XIX, Fig. 22, on left). In microtomed sections the curved tip may be cut away and produce a misleading appearance.

A wave of development passes from the tip of the ascogenous hypha downwards, so that the crozier developing from the top binucleate cell is at a more advanced stage than that developing from the second binucleate cell,

and the latter is farther on than that from the third (Pl. XIX, Fig. 20, and Text-fig. 6).

The top binucleate cell always forms a crozier, and the second and the third and possibly others frequently do so as well. Occasionally a binucleate cell does not grow out to form a crozier. Such cells are usually near the base of the ascogenous hypha. In Pl. XIX, Fig. 23, the cell above the basal bulb has four nuclei, presumably through mitosis of the nuclei of an originally binucleate cell (although no such mitosis has been observed). From the appearance of the nuclei it is not likely that this cell would develop further.

The height of the paraphyses in relation to the developing ascogenous hyphae is shown in Text-fig. 4, Pl. XIX, Fig. 17, and Pl. XIX, Fig. 20. In very feeble light-intensities they are relatively much taller in proportion to the ascogenous hyphae than in bright light. Nuclear divisions in the paraphyses are frequently seen. The spindles are not uniformly arranged and may be one behind the other in single file, obliquely placed (Pl. XIX, Fig. 20), or parallel (Pl. XIX, Fig. 21). The relative size of the nuclei in the paraphyses and ascogenous hyphae can be seen by comparing Pl. XIX, Fig. 21, and Pl. XIX, Fig. 14, which are drawn from the same material, and the relative size of the nuclei in the paraphyses and crozier from Pl. XIX, Fig. 20.

3. *Proliferation of the crozier and the formation of the ascus.*

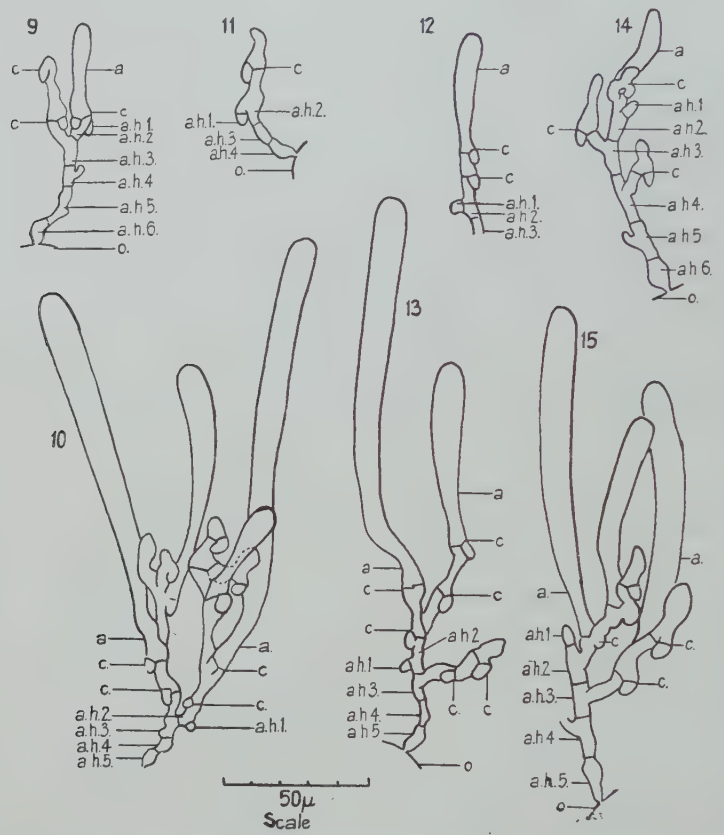
The binucleate penultimate cell of the crozier may form an ascus. In this case the two nuclei fuse (Pl. XIX, Figs. 26 (top crozier), 24). In addition to observation of stages in this fusion, further proof that such a fusion occurs is afforded by chromosome counts. There are twelve chromosomes on the metaphase plate at the mitotic division in the ascogenous hypha (Pl. XVIII, Fig. 13) and there are twelve bivalents in the fusion nucleus of the ascus undergoing the first division of meiosis (Pl. XIX, Fig. 27).

Instead of forming an ascus, the binucleate penultimate cell of the crozier may grow out to form another hook (Pl. XIX, Fig. 26). In this case the two nuclei in the penultimate cell of the first crozier do not fuse, the cell grows out to form a hook into which the nuclei pass one behind the other, and the two nuclei then undergo a simultaneous mitosis. As in the formation of the first crozier, walls are laid down across the spindle regions and the usual uninucleate terminal cell, binucleate penultimate cell, and uninucleate stalk cell are formed. The formation of croziers one above the other in this way may conveniently be termed 'erect' proliferation. The binucleate penultimate cell of the second crozier may form either an ascus or another crozier.

Sometimes the stalk and the terminal cell of the earlier, and occasionally of later, formed croziers take no further part in development (Pl. XIX, Fig. 26).

At other times, while the binucleate cell of a crozier is growing out into either an ascus or into another crozier, the uninucleate terminal cell and the uninucleate stalk cell anastomose (Pl. XIX, Fig. 22) and a new crozier is formed laterally from one or other of the fused cells. This 'lateral' proliferation is seen in Pl. XIX, Fig. 24.

Claussen (1912) describes another type of proliferation in which the binucleate penultimate cell of the first crozier forms *two* croziers (Claussen's Text-fig. 6, f_2–h_2). This type of proliferation has not been observed during the present investigation.



TEXT-FIGS. 9–15. Proliferation of ascogenous hyphae under different types of illumination. (All drawn from uncut material.) Hours after beginning of illumination stated. Fig. 9. Diffuse daylight+100-watt lamp at 6 inches, 12-hour day. After 33 hours. Fig. 10. Daylight, including sunlight, 12-hour day. After 49 hours. Fig. 11 Continuous illumination. After 33 hours (not most advanced stage). Fig. 12. Continuous illumination. After 36 hours. Fig. 13. Continuous illumination. After 48 hours. Fig. 14. Twelve hours' diffuse daylight and then continuous darkness. After 60 hours. Fig. 15. Diffuse daylight, 12-hour day. After 75 hours. *o* = oogonium, *a.h.* 1, *a.h.* 2, &c., successive cells of ascogenous hyphae from tip downwards, *c* = crozier, *a* = ascus.

In ascocarps developed in bright illumination, for example, with a 12-hour day of diffuse daylight+100-watt lamp at 6 inches, the shortness of the original ascogenous hypha may be compensated by a series of erect hooks, one above the other, before the first ascus is formed (Pl. XIX, Figs. 22, 26). This is even more strikingly the case if the illumination is continuous, day and night (Text-figs. 11, 12).

If grown in low light-intensities, such as diffuse daylight, the original ascogenous hypha is long and many-celled and an ascus is frequently formed from the first crozier of the top binucleate cell, though the second binucleate cell may sometimes form a second hook before giving rise to an ascus (Text-fig. 15).

In both high and low light-intensities 'lateral' proliferation through the union of the stalk and terminal cell of a crozier is usually delayed until the formation of the first ascus (Text-figs. 10, 14, 15; Pl. XIX, Fig. 24). Once this has occurred the process is likely to be repeated several times, the penultimate cell of each crozier forming an ascus and another lateral crozier being formed after the union of the terminal cell and stalk cell (Text-fig. 15).

Rarely, for example in continuous illumination, 'erect' proliferation may go on at the side of the first ascus instead of the more common 'lateral' proliferation (Text-fig. 13).

Light does not *directly* determine the method of proliferation, that is, whether an ascus or a crozier will be formed and whether 'lateral' proliferation will occur. With the normal diurnal alternation of light and dark periods it has been observed that any of these types of proliferation may take place in the dark though they appear to be determined by previous light treatment. The effect, as on earlier stages of development, is therefore indirect.

It must also be remembered that the asci and paraphyses must screen some of the light from the proliferating ascogenous hyphae at their base, and that the coloured pigments, so frequently found in the paraphyses of Discomycetes, may alter the wave-length of the light passing through them.

Discussion

The early stages in development

The position of origin of the ascogenous hyphae from the oogonium and of the branches of the ascogenous hyphae from the 'knob' both appear to be associated with the presence of two or more nuclei at those points.

As the branch grows out, two nuclei are frequently seen in a leading position near the tip. This is in agreement with the observations of Claussen (1907, 1912). It is notable that such pairs of nuclei have been seen also by such vigorous opponents of the Claussenian view as Moreau and Moreau (1930, Pl. II, Figs. 13, 14, 15, 18), Raymond (1934, Pl. XXXIV, Figs. 6, 8), and Gwynne-Vaughan and Williamson (1931, Pl. XIII, Figs. 24, 28, on right). The latter authors state, however, that such a paired arrangement is by no means universal and suggest that in their Pl. XIII, Fig. 28, it may be due to a recent nuclear division. This explanation is not supported by the present investigation, in which it has been found that only one mitosis occurs in the aseptate ascogenous hyphae and that this is immediately followed by septation. The presence of a leading pair of nuclei at the tip of the aseptate ascogenous hypha appears to be a real fact for which an explanation must be sought.

In the development of the germ-tube from the spore and in the development of the branch of an ascogenous hypha from the 'knob' there is a fundamental difference. In the spore, which has become multinucleate before the germ-tubes develop, the nuclei are irregularly arranged and there is not a pair of nuclei in a leading position in each germ-tube as it grows out (Pl. XIX, Fig. 28). The spore was originally uninucleate and therefore all the nuclei in the germinating spore are genetically alike.

Pyronema confluens will fruit in single-spore culture. Here, apart from the possibility of mutation, all nuclei must be alike. It has not been ascertained whether any paired arrangement of nuclei exists in the ascogenous hyphae of single-spore cultures. All figures in this paper are drawn from multispore cultures in which there is at least a possibility that nuclei of different genotype may be present.

There is evidence from other organisms that the association of nuclei of different genotype may stimulate growth. For example, Harder (1927) has shown that the dicaryotic, clamp mycelium of *Pholiota mutabilis* has a growth-rate approximately double that of either of the haploid mycelia from which it was built up and of the haploid mycelium formed by isolation of the penultimate cell of the dicaryotic mycelium, while this cell is uninucleate during clamp formation. The case of *Neurospora tetrasperma* is even more interesting. Here Dodge (1942) has shown that increased vigour, manifested by a greater growth-rate and more prolific formation of conidia results, not only by the combination of certain races of different 'sex' reaction, but also by the combination of certain races of the same 'sex' but of different genotype. Dodge suggests that this 'heterocaryotic' vigour may perhaps be accounted for on the same lines as Robbins' (1941) tentative explanation of 'hybrid' vigour (heterosis) in tomatoes, namely, by the ability of the hybrid to synthesize a full quota of vitamin-like growth substances.

But is there any evidence that the oogonium which produces the ascogenous hyphae of *Pyronema confluens* contains nuclei of different genotype? If such nuclei were present and their association stimulated growth, what effect would be produced? To answer the second of these questions first, it would explain why the ascogenous hyphae arise at certain points on the oogonium, always near two or more nuclei. It would explain also how, after being mixed up in the 'knob' owing to the entry of nuclei one at a time, two nuclei of different genotype could be re-isolated. By first stimulating the formation of a branch, and secondly by stimulating the rate of growth of that branch, the two nuclei would be carried away from the rest.

As to whether the oogonium of *P. confluens* at the time of formation of the ascogenous hyphae contains nuclei of different genotype, the evidence is conflicting and has not been specifically re-investigated in the present work. For all exponents of the Dangeardian view the nuclei which enter the ascogenous hyphae are haploid and of ascogonial origin. According to Harper (1900) and Gwynne-Vaughan and Williamson (1931) they are diploid as a result of sexual fusion, while according to Claussen (1912) the male and female nuclei are

associated in pairs. On any of these views, whether the nuclei entering the ascogenous hyphae are genetically alike or not will depend on the type of nuclei present in the young, multi-nucleate sex organs at their inception, and this in turn will depend on such factors as the mutation rate, and the presence or absence of anastomoses between mycelia from different spores when grown in multispore culture. If antheridial nuclei pass into the oogonium, this would obviously increase the chance of associating nuclei of different genotype, especially in multispore cultures and if the sexual organs arise from the dichotomy of more than one hypha. These matters are, however, still controversial.

It is well known that cultures of *P. confluens*, like those of many other fungi, deteriorate after some years of intensive cultivation. It may be that the speculative suggestion made here of the possible stimulating effect of the association of nuclei of different genotype at the origin of the ascogenous hyphae and their branches applies only to cultures after they have undergone mutations involving loss of ability to synthesize certain growth substances, or in which selection of these factors has occurred.

Thus observations on the young ascogenous hyphae raise problems which can only be solved by critical re-examination of earlier stages of development, supplemented perhaps by biochemical and genetical studies.

The nuclear division in the ascogenous hypha and the crozier

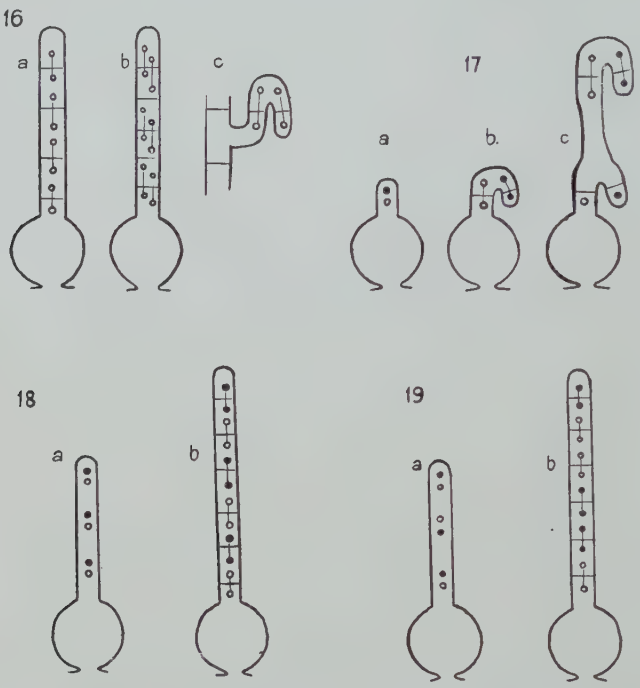
The present investigation confirms the work of Gwynne-Vaughan and Williamson (1931) on the method of nuclear division in the ascogenous hyphae and is in disagreement with that of Claussen (1912).

The mitosis in both the ascogenous hypha and the crozier is essentially similar and consists in the simultaneous division of nuclei arranged in single file. In both, walls are formed as ingrowing rings across the spindles and the arrangement of the nuclei in the resulting cells is 1:2:1 in the crozier and 1:2:2 . . . 1 (or more, because of the basal swelling) in the ascogenous hypha. The parallel spindles in the crozier, superficially resembling the Claussenian scheme, are the result of the hooked form (Text-fig. 16*a*, *b*, *c*).

The end cell of the ascogenous hypha does not form a crozier. This fact can be verified easily in a 'squash' preparation of an ascocarp at the right stage of development. The reason why it does not form a crozier is that it is uninucleate. The only instance in which it was seen to play any further part in development was in an exceptionally short ascogenous hypha when it fused with the basal cell, as frequently happens in a crozier. Claussen's figures (1912) show two very small and apparently degenerating nuclei in the end cell, but he states that the end cell may form a crozier. His Taf. 4, Fig. 56, showing this could equally well be interpreted as lateral proliferation as in his Text-fig. 7, *g*, although in either of these interpretations the wall at the base of one of the croziers is unexplained.

How can Claussen's Taf. 4, Fig. 43, of conjugate nuclear division be explained? This figure shows a single pair of nuclei at telophase, but no

indication is given as to the position of the pair of nuclei along the length of the ascogenous hypha, and so short a piece of the hypha is drawn that one cannot even be certain whether it is septate or not. Gwynne-Vaughan and Williamson (1931) suggest that it might be a crozier division seen from an



TEXT-FIGS. 16–19. Fig. 16. Comparison of mitosis in ascogenous hyphae and crozier: *a*, simultaneous division of nuclei in single file (as in Gwynne-Vaughan and Williamson, 1931); *b*, conjugate mitosis (as in Claussen, 1912); *c*, mitosis in crozier. Fig. 17*a*, *b*, *c*. Effect of simultaneous mitosis in single file with one pair of nuclei of different genotype in branch of ascogenous hyphae. *c*, with crozier. Fig. 18*a*, *b*. Effect of simultaneous mitosis in single file of three pairs of nuclei of different genotype, the two nuclei in each pair similarly orientated. Result, all binucleate cells contain two nuclei of different genotype. Fig. 19*a*, *b*. Effect of simultaneous mitosis of three pairs of nuclei of different genotype when the two nuclei in each pair are not similarly orientated. Result, top binucleate cell has nuclei of different genotype, but some of the lower binucleate cells may contain two similar nuclei.

angle which obscures the hook, and Martens (1946) agrees that this is feasible. Other possible explanations are that it occurred near the base of an ascogenous hypha as in Pl. XVIII, Fig. 14, or that it took place in a binucleate cell which became quadrinucleate without forming a crozier as in Pl. XIX, Fig. 23. It is hardly likely that it could have been confused with a paraphysis division owing to the smaller size of the nuclei here, but at telophase the difference is not so marked as in earlier stages of division (Pl. XVIII, Fig. 15, and Pl. XIX, Fig. 21).

Although many workers on other Ascomycetes have supported Claussen's theory, remarkably few have figured the conjugate nuclear division which is an

integral part of it. Martens (1946) in his masterly and comprehensive survey of the whole field can only muster seven examples. Of these, *Galactinia succosa* (Maire, 1905) is the most convincing, and two, *Sclerotinia gladioli* (Drayton, 1934) and *Phyllactinia corylea* (Colson, 1938), must certainly be rejected, the former because, in the figures cited, it is doubtful whether the nuclei are dividing and in at least one case the hypha is already septate, and the latter because the nuclei are in single file during division and their spindles only appear to be parallel because of the curved form of the hypha. Martens himself concludes (loc. cit., p. 266): 'Ainsi, sur ce point essentiel, crucial, du schéma claussenien, nous devons enregistrer une *carence presque complète de documents inéquivoques*.'

The simultaneous division of a single row of nuclei has not been figured frequently either. It is shown in *Humaria granulata* (Gwynne-Vaughan and Williamson, 1930), *Ascobolus magnificus* (Gwynne-Vaughan and Williamson, 1932), *Ascophanus aurora* (Gwynne-Vaughan and Williamson, 1934), and *Phyllactinia corylea*, at two stages in development (Colson, 1938). The uninucleate terminal cell and following binucleate cells are clearly figured in an early paper on *Peziza* (*Pustularia*) *catinus* (Guilliermond, 1905), confirmed (without nuclear details) by Chadefaud (1943) and in *Aleuria* (Aldinger, 1936), but in these species the binucleate penultimate cell is said to form an ascus directly.

The method of nuclear division and septation in the ascogenous hyphae does not have any important bearing on the validity of either the Dangeardian or the Harperian theory, but for the Claussenian interpretation its implications are far-reaching. If the view of nuclear division supported in the present investigation proves correct, the continuing association of a male and a female nucleus, or their descendants, until their final fusion in the ascus, demanded on the Claussenian theory, can only be achieved in one of two ways:

(i) If the ascogenous hypha is exceptionally short and contains only a single pair of nuclei, the descendants of these nuclei can be kept in pairs by forming a series of croziers one above the other, after the simultaneous division of the nuclei in the ascogenous hypha itself (Pl. XIX, Fig. 22, and diagrammatically in Text-fig. 17*a*, *b*, *c*).

(ii) If the ascogenous hypha contains more than one pair of nuclei, the male and female nuclei of each pair must be similarly orientated in each pair as shown in Text-fig. 18. If they are not (Text-fig. 19), some of the binucleate cells below the first will contain nuclei of the same sex (Wilson, 1938; Chadefaud, 1943).

In relation to the second alternative, Claussen (1912) says that there is a difference in the size of the male and female nuclei in each pair in *P. confluens*, but unfortunately this cannot be detected with certainty in his figures and no other author has found any such difference in size of the nuclei at this stage. In *Ascobolus strobilinus* (Schweizer, 1931), where there is a marked difference in the size of the two nuclei in each pair, the figure given shows only the

base of the ascogenous hypha and is indecisive in regard to the problem under discussion.

It has been claimed by Chadefaud (1943) that, in *Pustularia catinus*, cells containing two nuclei of the same kind remain sterile. In *Pyronema confluens* the evidence is that frequently all the binucleate cells form croziers; if one does not, it is generally at the base of a branch. The bulk of genetical work on the Ascomycetes supports the view that nuclei of different genotype fuse in the ascus, but in *Glomerella cingulata* some asci resulting from the fusion of two genetically similar nuclei have been recorded (Chilton and Wheeler, 1949). It is suggested that genetical work on *Pyronema* might settle some of the issues raised here.

The asci and the proliferation of the crozier

All observers are agreed that a nuclear fusion in the penultimate cell of the crozier precedes the formation of the ascus, and the proliferation of the crozier, so clearly described by Claussen (1912), is substantially confirmed here and has been observed in many other Ascomycetes.

The chromosome counts in the ascogenous hypha, crozier, and in the first division of the ascus recorded here are in agreement with those of Claussen (1912) and Gwynne-Vaughan and Williamson (1931), and, for the first division in the ascus, with Moreau and Moreau (1931), Colson (1942), and Hirsch (1950). They differ from those of Harper (1900), Dangeard (1907), Brown (1909, 1915), and Raymond (1934).

The effect of light

The object of using different types of illumination was to find out whether such a cultural condition could account for the great differences in the published figures of the ascogenous hyphae. To some extent this object has been achieved, and one might venture to guess, for example, that Pl. XIII, Figs. 33 and 35, of Gwynne-Vaughan and Williamson (1931) are drawn from material grown in bright light, and that Pl. II, Figs. 19–21, of Moreau and Moreau (1930) are drawn from material developing rather slowly in a lower light-intensity.

As a side issue it has been found that the type of illumination (using this term in a broad sense to include the effects of both light-intensity and of temperature) affects the rate of development and the form of the ascogenous hypha and its method of proliferation. In diffuse daylight, and in low light-intensities in general, the trichogyne may fail to connect with an antheridium, thus leading to the formation of sclerotia instead of ascocarps. Robinson (1926) has suggested that 'the effect of the light is to produce a photochemical modification in some substance in the mycelium which arrests the tendency towards fatty and other degenerative changes'. The indirect effects of light recorded here, and also the more detailed results of Kerl (1937), might also be explained by a similar hypothesis, such as the action of some substance

which requires a photochemical stage in the sequence of its synthesis, but the problem has now reached the stage when it needs precise biochemical investigation.

Finally, the effects of light on the ecology of the fungus may be briefly considered. In diffuse daylight a large proportion of the nuclei issuing from the oogonium are utilized in the formation of croziers and asci. In bright light, relatively few are used, but a large number of asci may be formed in the end, owing to the effects of lateral proliferation. *Pyronema confluens* is well adapted, therefore, to a wide range of light intensity and has been seen growing well on the site of old bonfires in almost full sunlight, but more often in the partial shade of trees.

P. confluens has generally been recorded in the summer and especially in the autumn months. Personal records range from June to October, and information kindly supplied by Mrs. F. L. Balfour-Browne on herbarium material in the British Museum (Natural History) shows that specimens were collected in every month from April to October, mostly in September and October, in Britain, Europe, and America (W. Virginia). Although its prevalence in autumn is perhaps related to its habitat on burnt ground, the importance of suitable conditions of light and temperature for efficient fruiting must play an important part in its seasonal range.

Literature Cited

Aldinger, L., 1936: Cytological Phenomena in *Aleuria* sp. Amer. Journ. Bot., xxiii. 639–44.

Brown, W. H., 1909: Nuclear Phenomena in *Pyronema confluens*. John Hopkins Univ. Circ., No. 217. 42–45.

—— 1915: The Development of *Pyronema confluens* var. *inigneum*. Amer. Journ. Bot., ii. 289–98.

Buller, A. H. R., 1933: Researches on Fungi, v. Longmans.

Chadefaud, M., 1943: Sur les divers types d'éléments dangeardiens des Ascomycètes. Rev. scient., Paris, No. 3217, 77–80.

Chilton, S. J. P., and Wheeler, H. E., 1949: Genetics of *Glomerella*, VI. Linkage Amer. Journ. Bot., xxxvi. 270–3.

Claussen, P., 1907: Zur Kenntnis der Kernerverhältnisse von *Pyronema confluens*. Ber. d. Deut. Bot. Gesell., xxv. 586–90.

—— 1912: Zur Entwicklungsgeschichte der Ascomyceten. *Pyronema confluens*. Zeitschr. f. Bot., iv. 1–64.

Colson, B., 1938: The Cytology and Development of *Phyllactinia corylea* Lév. Ann. Bot., n.s., ii. 381–402.

—— 1942: The Chromosome Cycle in Two Common Fungi. Journ. Queckett Micr. Club, Ser. 4, i. 205–16.

Dangeard, P. A., 1907: Recherches sur le développement du périthèce chez les Ascomycètes. Le Botaniste, x. 1–385.

Dodge, B. O., 1942: Heterocaryotic vigor in Neurospora. Bull. Torrey Bot. Club, lxix. 75–91.

Drayton, F. L., 1934: The Sexual Mechanism of *Sclerotinia gladioli*, Mycologia, xxvi. 46–72.

Godward, M. B. E., 1948: The Iron Alum Acetocarmine Method for Algae. Nature, clxi. 203.

Guilliermond, A., 1905: Remarques sur la karyokinèse des Ascomycètes. Ann. Myc., iii. 343–61.

GWYNNE-VAUGHAN, H. C. I., and WILLIAMSON, H. S., 1930: Contributions to the Study of *Humaria granulata* Quèl. Ann. Bot., xliv. 127–45.
—— —— 1931: Contributions to the Study of *Pyronema confluens*. Ibid., xlv. 355–71.
—— —— 1932: The Cytology and Development of *Ascobolus magnificus*. Ibid., xlvi. 653–70.
—— —— 1934: The Cytology and Development of *Ascophanus aurora*. Ibid., xlviii. 261–72.
HARDER, R., 1927: Zur Frage nach der Rolle von Kern und Protoplasma in Zell geschehen und bei der Übertragung von Eigenschaften. Zeitschr. f. Bot., xix. 337–407.
HARPER, R. A., 1900: Sexual Reproduction in *Pyronema confluens* and the Morphology of the Ascocarp. Ann. Bot., xiv. 321–400.
—— 1905: Sexual Reproduction and the Organization of the Nucleus in Certain Mildews. Publ. Carnegie Inst. Washington, xxxvii.
HIRSCH, H. E., 1950: No Brachymeiosis in *Pyronema confluens*. Mycologia, xlii. 301–5.
KERL, I., 1937: Über Regenerationsversuche an Fruchtkörpern und andere entwicklungsphysiologische Untersuchungen bei *Pyronema confluens*. Zeitschr. f. Bot., xxxi. 129–74.
KIHLMAN, O., 1885: Zur Entwickelungsgeschichte der Ascomyceten. Acta Soc. sc. Fennicae, xiv. 337–51.
MAIRE, R., 1905: Recherches cytologiques sur quelques Ascomycètes. Ann. Myc., iii. 123–54.
MARTENS, P., 1946: Cycle de développement et sexualité des Ascomycètes. La Cellule, I (2), 125–310.
MOREAU, F., and MOREAU, Mme, 1930: Le Développement du périthèce chez quelques Ascomycètes. Rev. Gén. Bot., xlii. 65–98.
—— —— 1931: Existe-t-il une double réduction chromatique ches les Ascomycètes? Ibid., xliii. 465–73.
RAYMOND, J. R., 1934: Contribution à la connaissance cytologique des Ascomycètes. Le Botaniste, xxvi. 371–538.
ROBBINS, W. H., 1941: Growth of Excised Roots and Heterosis in Tomato. Amer. Journ. Bot., xxviii. 216–25.
ROBINSON, W., 1926: The Conditions of Growth and Development of *Pyronema confluens* Tul. (*P. omphalodes* (Bull.) Fuckel). Ann. Bot., xl. 245–72.
SCHWEIZER, G., 1931: Zur Entwicklungsgeschichte von *Ascobolus strobilinus* nov. spec. Planta, xii. 588–602.
WILSON, I. M., 1938: The Cytology of Some Higher Fungi. Ph.D. Thesis, Univ. London.

EXPLANATION OF PLATES XVIII AND XIX

Illustrating Dr. Irene Wilson's paper on 'The Ascogenous Hyphae of *Pyronema confluens*'.

PLATE XVIII

All figures are drawn × 1,450

FIG. 1. Oogonium cut near surface showing origin of enucleate ascogenous hyphae. Trichogyne contents disorganized. Antheridium cut and appearing empty. Opening between antheridium and trichogyne.

FIG. 2. Oogonium cut in median section showing nuclei in peripheral cytoplasm and enucleate ascogenous hyphae arising opposite groups of nuclei.

FIGS. 3, 4, 5. Passage of nuclei into ascogenous hyphae one at a time. Deeply staining remnants of disorganized contents of antheridium and trichogyne in Fig. 5. Opening between trichogyne and antheridium remains.

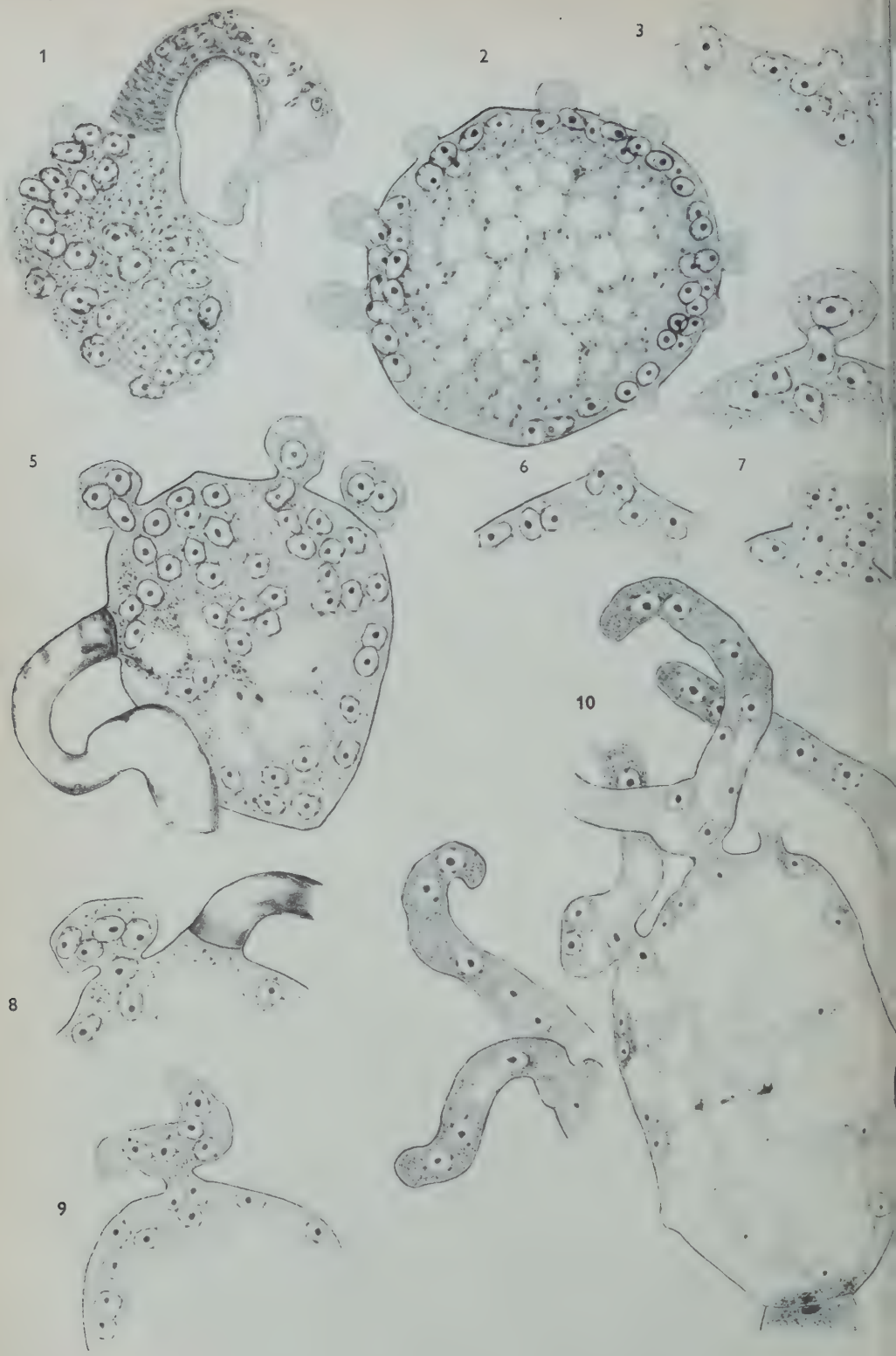
FIG. 6. Two nuclei passing into ascogenous hypha at same time (unusual).

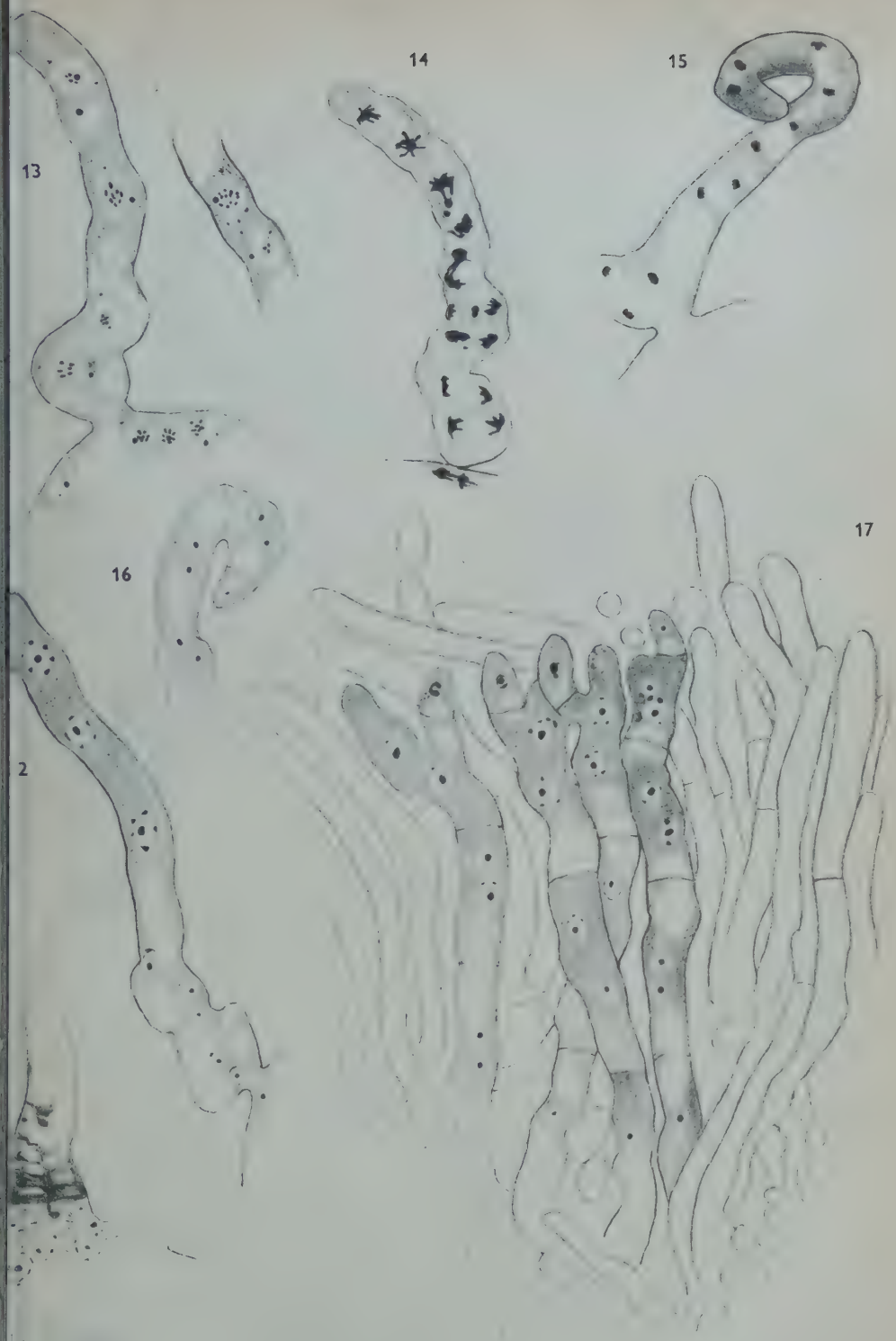
FIG. 7. 'Knob' with wide opening at base (unusual).

FIG. 8. Normal 'knob' with narrow opening at base and containing 4–5 nuclei.

FIG. 9. Branches beginning to grow out, each opposite a pair of nuclei. Nuclei still crowding into ascogenous hypha from oogonium.

FIG. 10. Oogonium with aseptate ascogenous hyphae, a pair of nuclei in a leading position in each. Pore plugs on wall between oogonium and stalk cell.


1
2
3
5
6
7
10
8
9


13
14
15
16
17
2

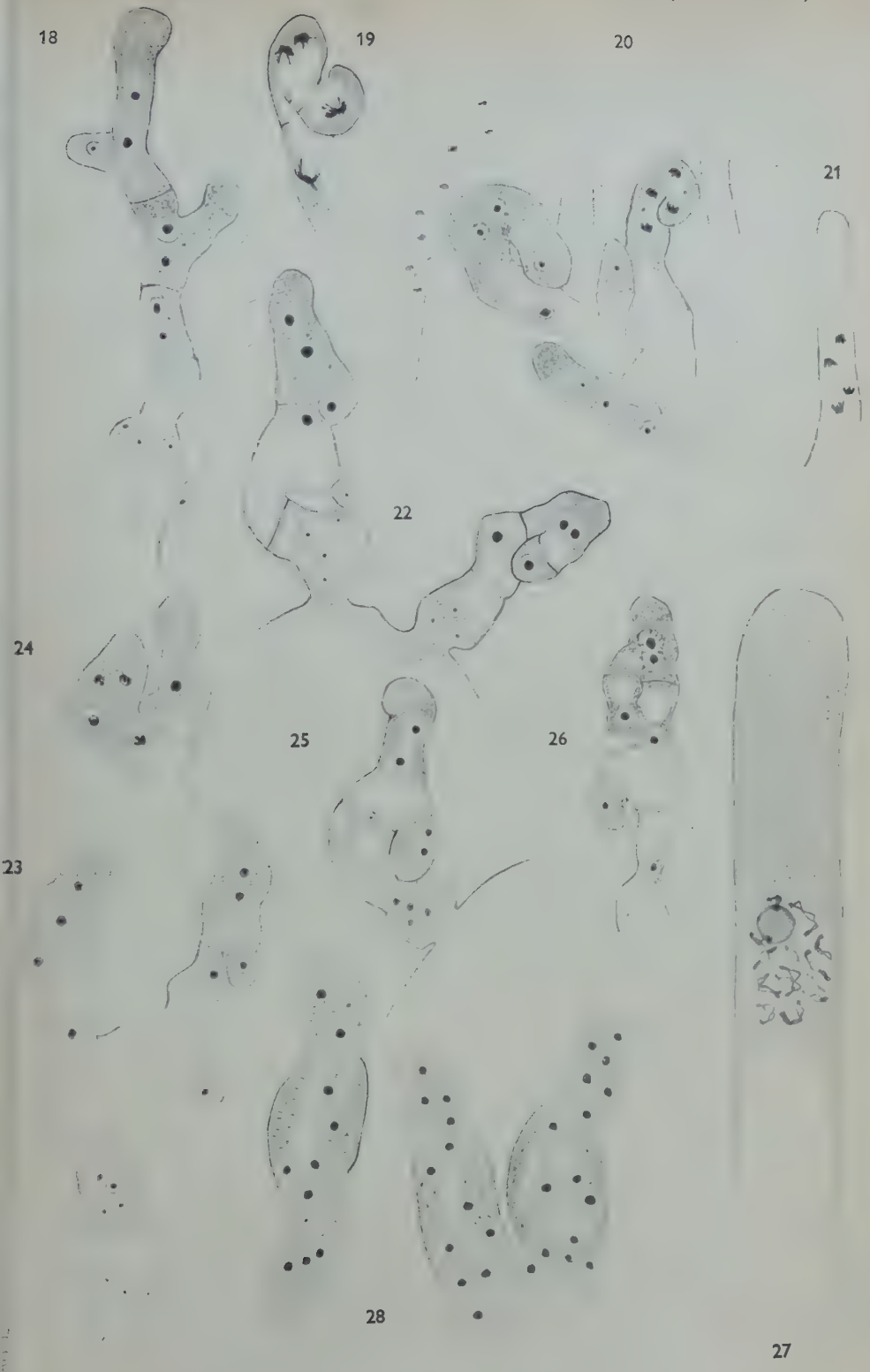

18
19
20
21
22
24
25
26
23
28
27

FIG. 11. Perforation in wall between oogonium and trichogyne and dark trichogyne material passing through it into oogonium. Oogonium has ascogenous hyphae developing.

FIG. 12. Nuclei in branch of ascogenous hypha well spaced out and in single file. Smaller and irregularly arranged nuclei in basal swelling.

FIG. 13. Simultaneous mitosis of nuclei in ascogenous hyphae and oogonium. Twelve chromosomes on metaphase plate (top nucleus on right).

FIG. 14. Late anaphase of simultaneous mitosis in ascogenous hypha and oogonium. Dividing nuclei remain in single file in narrow branch, irregular, or even parallel, at base.

FIG. 15. Telophase of mitosis in ascogenous hypha. Nuclei still in single file in narrow branch.

FIG. 16. Daughter nuclei resulting from mitosis separated into a uninucleate end cell, followed by a series of binucleate cells, by ingrowing septum across spindle regions, now marked by vacuoles.

FIG. 17. Long, septate ascogenous hyphae with uninucleate end cell and series of binucleate cells with large nuclei. Top binucleate cell has started to grow out. Paraphyses indicated in outline only.

PLATE XIX

All figures are drawn × 1,450

FIG. 18. Septate ascogenous hypha. The two upper binucleate cells are beginning to grow out to form croziers. Nuclei, in uninucleate tip cell and in base, small.

FIG. 19. Late anaphase of simultaneous mitosis of two nuclei in crozier. Wall beginning to form across spindle regions as ingrowing ring.

FIG. 20. Ascogenous hypha with uninucleate terminal cell and three stages in the development of croziers from the next three (originally binucleate) cells. Paraphyses indicated in outline, with nuclear division in paraphysis on left.

FIG. 21. Paraphysis division with parallel spindles.

FIG. 22. On right, short curved ascogenous hypha developed in very strong illumination and resembling crozier except that basal cell is multinucleate. On left, binucleate cell of original ascogenous hypha has formed a crozier whose terminal cell is now anastomosing with the stalk cell.

FIG. 23. The cell above the basal bulb is quadrinucleate but is not forming a crozier.

FIG. 24. The two nuclei in the penultimate cell of a crozier have fused to form the definitive nucleus of the young ascus. The stalk and terminal cells have anastomosed and are now growing out laterally to form another crozier.

FIG. 25. A very short ascogenous hypha as in Fig. 22. A nucleus from the basal cell has passed into the end cell after anastomosis.

FIG. 26. Erect proliferation by the formation of a crozier from the penultimate cell of the first crozier. Nuclear fusion in penultimate cell of second crozier.

FIG. 27. Late diplotene of the first division of meiosis in the ascus. Twelve bivalents are present.

FIG. 28. Three germinating ascopores showing arrangement of nuclei during outgrowth of germ tubes.

The Strength of Banana Petioles in relation to Ploidy

BY

N. W. SIMMONDS

(*Banana Research Scheme, Botany Department, Imperial College of Tropical Agriculture, Trinidad, B.W.I.*)

Abstract

The strength of the banana petiole has been measured as the breaking strength corrected by covariance for the weight of the lamina it bears. Strength is less in triploids (as compared with diploids) and still less in tetraploids, thus agreeing with the field observation that polyploids have weaker leaves than diploids. There are also differences between plants of different specific origins. Subsidiary results concern the weight of the leaf and the leaf index (length/breadth) in relation to ploidy.

1. Introduction

THE leaf of *Musa* is one of the largest to be found in any plant and may attain about 2 square metres in area and over a kilogram in weight. The strains imposed on the petioles must be great, but nevertheless the leaf of the ordinary wild diploid banana is mechanically nicely adjusted to its environment, for breakage at the petiole, even in high winds, is rarely or never seen; instead, the lamina tears and the leaf remains in position even if tattered (cf. Skutch, 1927). In polyploids, however, this mechanical adjustment is lost, the leaves droop more than in diploids and are more liable to breakage of the petiole near the base of the lamina. Flaccidity of the petiole is roughly proportional to ploidy (up to the pentaploid level, at least) and is negatively correlated with dry matter content (Simmonds, 1948*b*). Fragility—i.e. liability to actual breakage as distinct from mere excessive bending—has now been measured and the results are described in this paper. Subsidiary results concern the weight of the leaf and the leaf index in relation to ploidy.

2. Materials and Methods

Plant materials were the collections of diploid and polyploid bananas maintained or bred at this Institution. All this work has been based on a series of diploid, triploid, and tetraploid clones of three distinct origins in Eumusa, namely, *Musa acuminata* Colla, *Musa Balbisiana* Colla, and hybrids between these species. The diploids used were the wild species themselves and synthesized hybrids between them; triploids were established edible varieties whose origins were determined by an examination of phenotypes (Dodds and Simmonds, 1948); tetraploids were derived from pollination of edible triploids. Wherever possible, several clones were used in each category of ploidy,

[Annals of Botany, N.S. Vol. XVI, No. 63, July, 1952.]

but sometimes abundant material of more than one or two clones was not available. Pentaploids were not included in this study since material was sparse.

Breaking strengths of petioles were measured by means of a form of steelyard made from an L-section steel girder 2 metres long. The girder was graduated in decimetres and nicks were cut at suitable intervals so that it might be placed accurately upon a knife edge as a fulcrum. The fulcrum was borne in slots on the edge of a long narrow wooden box in which the petiole was held under two transverse wooden bars, the nearer faces of which were 30 cm. apart. The girder was pierced at one end and a cord (protected by a piece of rubber tubing) served to attach it to the petiole under test. The apparatus allowed for adjustment of the petiole-fulcrum distance to 10, 15, 20, 30, 40, 50, and 60 cm.; this distance was selected by experience (a little practice was necessary) and a kilogram lead weight with a wire hook was suspended from the girder and slid in a distal direction until the petiole broke. Care was taken to keep the rate of movement of the weight reasonably constant (roughly 20 cm. per second). The end point was sharp, the petiole snapping suddenly after a preliminary 'creaking'. Graphs of distances and breaking strengths were constructed from a knowledge of weight and length of girder.

The portion of the petiole tested was the same as in the previous study, namely, the 30-cm. length which had its centre at the base of the lamina.

3. Results

1. *Leaf weight*

The tendency of a petiole to bend and break depends not only on the strength of the petiole but also on the strain imposed upon it. Accordingly, measurements of length, breadth, and weight of ten leaves from each of nine categories of plant were made. The nine categories were diploids, triploids, and tetraploids of origins *M. acuminata*, *M. Balbisiana*, and the hybrid between them. The product length × breadth gives a good measure of leaf area (Simmonds, 1948*b*), and this was used as a covariate in the analysis of weight:

Item.	Degrees of freedom.	Mean square.	Variance ratio.	*P.*
Species	2	20,214·2	5·54	0·1–1%
Ploidy	2	61,556·1	16·86	0·1%
Sp × Pl	4	1,418·6	—	—
Error	80	3,651·7	—	—
Deviations . . .	72	1,638·0	—	—
Differences . . .	8	21,774·5	13·3	0·1%

Species and ploidy are both significant but their interaction is not. There are also differences between the nine within-treatment regressions of weight on area, the main effects for species and ploidy being highly significant (variance ratios respectively 5·63 and 21·02 for $N_1 = 2$ and $N_2 = 84$) while the

interaction is not. Table I shows that leaf weight rises from diploidy to tetraploidy, the triploid-tetraploid rise being consistently greater than that between diploid and triploid. *M. Balbisiana* at all levels of ploidy has heavier leaves than *M. acuminata* and the hybrid is roughly intermediate. Correlations between area and weight within groups are consistently high and positive; regressions show a distinct upward trend from diploidy to tetraploidy, and also a slight (but significant) rise from *M. acuminata* through the hybrid to *M. Balbisiana.*

TABLE I

Weights of Banana Leaves. Mean Weights (W, g.) adjusted by Covariance for Leaves 1 sq. m. in Area; Correlation of Weight with Area (r); and Regressions of Weight on Area (b as g. per cm.²)

		Acuminata.	Hybrid.	*Balbisiana.*	Means.
Diploid	*W*	412	434	467	438
	r	+0·9812	+0·9956	+0·9974	—
	b	+0·03875	+0·03858	+0·05080	+0·04774
Triploid	*W*	454	462	486	467
	r	+0·9752	+0·9851	+0·9945	—
	b	+0·04691	+0·05170	+0·05194	+0·05051
Tetraploid	*W*	487	537	557	527
	r	+0·9903	+0·9913	+0·9876	—
	b	+0·05144	+0·06546	+0·06911	+0·06497
Means	*W*	451	478	503	—
	b	+0·04478	+0·05643	+0·05738	—

Least significant difference between means (5%) = 53 g. (different means have, strictly speaking, different standard errors, but the adjustment here seems small and has been neglected).

2. *Breaking strength in relation to leaf weight*

Correlations between leaf area and weight are consistently very high (Table I). Hence weight of a leaf can be estimated rather accurately from a knowledge of its area (actually the product length×breadth), ploidy, and specific origins. This was done for samples of ten leaves, for each of which the petiole strength was known and the data analysed with the interpolated leaf weight as a covariate, thus:

Item.	Degrees of freedom.	Mean square.	Variance ratio.	*P.*
Species	2	38·6	9·2	< 0·1%
Ploidy	2	187·9	44·7	< 0·1%
Sp×Pl	4	12·3	2·9	1–5%
Error	80	4·2	—	—
Deviations	72	3·1	—	—
Differences	8	14·2	4·6	< 0·1%

Species and ploidy are highly significant, their interaction probably so. There are also real differences between regressions.

Results are summarized in Table II. All three specific categories show a decline in strength with increasing ploidy, the drop between diploid and triploid being greater than that between triploid and tetraploid, as was found in the case of leaf posture (Simmonds, 1948*b*). Differences between species are most marked at the diploid level where the hybrid significantly exceeds *M. Balbisiana* which itself exceeds *M. acuminata*; the hybrids seem to be less affected by tetraploidy than their parents. These results are in very good accord with field knowledge of bananas, for tetraploid strains of *M. acuminata* and *M. Balbisiana* are the most liable to breakage in the field. We can probably say that a breaking strength of 8–9 kg. for a leaf weighing 400 g. represents a threshold over which the leaf runs risk of breakage.

TABLE II

Mean Breaking Strengths (S in kg.) of Banana Petioles adjusted by Covariance for a standard Leaf Weight of 400 g.; with Coefficients of Correlation (r) and Regression (b as kg. per 10 g.) of Breaking Strength on Leaf Weight

		Acuminata.	Hybrid.	*Balbisiana.*	Means.
Diploid	*S*	10·84	15·44	13·75	13·35
	r	+0·9756	+0·9176	+0·9780	—
	b	+0·3345	+0·3358	+0·4224	+0·3941
Triploid	*S*	9·36	10·72	10·43	9·61
	r	+0·9416	+0·9733	+0·9790	—
	b	+0·3380	+0·3868	+0·3383	+0·3504
Tetraploid	*S*	7·82	9·01	7·63	8·25
	r	+0·9787	+0·9724	+0·9659	—
	b	+0·2412	+0·2924	+0·2206	+0·2579
Means	*S*	9·43	11·72	10·62	—
	b	+0·2777	+0·2834	+0·2793	—

Least significant difference between mean breaking strengths (5%) = 1·83 kg. (see note to Table I).

In view of the practical importance (see Discussion) which attaches to comparison between triploids and tetraploids of *M. acuminata*, more extensive data on these two categories were obtained and analysed. Thirty leaves of each were taken and the results were (abbreviations, *S* and *b*, as in Table II):

	S	*b*
Triploid *acuminata* . . .	9·69	0·3548
Tetraploid *acuminata* . . .	8·37	0·2546
Variance ratio	5·18	24·33
P	5%	< 0·1%

3. *Leaf index*

The data used in the analysis of leaf weight described above were used to provide information on the leaf index, the ratio of length to breadth. Analysis of variance of this index, corrected as before by the use of length × breadth as a covariate, showed highly significant effects for species, ploidy, and inter-

action; the regression of leaf index on area was significant and positive, but there was no evidence of difference between the nine individual regressions, all of which (like the correlation coefficients) were positive though not all were significant. Table III summarizes the results. Leaf index apparently falls as ploidy rises in the *M. acuminata* series but rises with ploidy in *M. Balbisiana*. It shows no perceptible trend in the hybrids and indeed in the diploids it far exceeds the larger parent, while in the triploids it is less than the smaller one. Re-examination of the data used for the analysis of breaking strengths gave similar results.

TABLE III

Leaf Index (I as length/breadth) of Banana Leaves. Means (adjusted by Covariance for Leaves 1 sq. m. in Area) and Coefficients of Correlation (r) between Area and Leaf Index. In brackets, supplementary data on the leaf index (see text)

		Acuminata.	Hybrid.	*Balbisiana.*
Diploid	I	4·10 (3·63)	5·37 (4·23)	3·14 (3·57)
	r	+0·7839*	+0·7799*	+0·9499*
Triploid	I	3·85 (3·76)	3·51 (3·58)	3·68 (3·49)
	r	+0·4000	+0·6883*	+0·7927*
Tetraploid	I	3·54 (3·53)	3·56 (3·54)	4·15 (4·01)
	r	+0·5517	+0·8722*	+0·5938

Least significant difference (5%) = 0·49 (see note to Table I). * Significant at the 5% level.

In order to examine the possibility that leaf index falls with rising ploidy in *M. acuminata* but rises in *M. Balbisiana* another series of measurements were made and the results are given in brackets in Table III. Each entry is the mean leaf index (adjusted for leaf size) of five leaves from each of at least three clones, so far as possible different from the clones used previously. The results are not inconsistent with the previous figures, for in each case the four highest values are for the hybrid diploids, the *Balbisiana* tetraploids, and the *acuminata* diploids and triploids.

Differences between clones were considerable. For example, values for seven diploid hybrids ranged from 3·67 to 5·11; for three acuminata tetraploids from 2·49 to 4·13, and so forth. It is also known (unpublished) that there are considerable differences between members of the Cavendish complex of edible bananas, a series of closely related clones differentiated by somatic mutation. Here the mutants differ in elongation of leaf structures and so leaf index increases with increasing stature of the plant.

It is concluded that the effect of ploidy on leaf index is not a simple one and that in any case it is obscured by large genetic differences between clones. Presumably a component of the effect of ploidy is the differential restriction of development of the two lamina-halves of the banana leaf (Simmonds, 1948*b*).

4. Discussion

The angle at which the leaves of diploid and polyploid bananas are borne has been shown to be correlated with dry matter content of the petioles (Simmonds, 1948*b*), and it was suggested that the drooping flaccid leaves of polyploids were weaker or, at least, more liable to breakage in the field than the stiffer leaves of diploids. The present work shows that this is so, that the results of laboratory measurement agree very well with views formed in the field. Since this work was completed the writer has been able to examine the effects of a hurricane on banana fields in Jamaica. The Banana Breeding Station at Bodles on the St. Catherine plain suffered severe damage, since it lay near the actual track of the storm. It was particularly interesting to see therefore that triploid and tetraploid acuminatas were utterly destroyed by the wind, but that a few of the smaller pseudostems of diploid *M. Balbisiana* (which is used as a windbreak along exposed edges of the fields) were still standing and even bore erect though shredded leaves.

This is an extreme case; but many banana-growing areas suffer high winds in the summer and a marked susceptibility to leaf breakage is an undesirable agricultural character. The standard varieties of banana at present in use are disease-susceptible triploid acuminatas and the breeding programme seeks to replace them by disease-resistant tetraploids. It is worthwhile therefore to compare them rather closely with regard to leaf strength. From Table I and data presented in section 3 above we have:

Leaf area (sq. m.)		0·7	1·0	1·3	1·6	1·9
Triploid	*W* (g.)	313	454	595	736	877
	S (kg.)	6·43	11·44	16·44	21·44	26·44
Tetraploid	*W* (g.)	333	487	641	795	949
	S (kg.)	6·78	10·70	14·62	18·54	22·46
S (Triploid)/*S* (Tetraploid)		0·95	1·07	1·13	1·16	1·18

The larger the leaf, the greater the disparity between the two; this arises, of course, from the occurrence in tetraploids of a high weight-area regression (Table I) and a low strength-weight regression (Table II). Thus, the larger the leaf, the greater the risk of breakage in tetraploids relative to triploids. Field trials alone can tell how important this is. Indications at present are that the tetraploids do not suffer appreciably more severely than triploids under normal conditions; but it is obvious that they lie near a threshold over which they may pass through the agency of wind or, perhaps, excessive nitrogenous manuring. However, it may be that the tetraploids are inherently sufficiently vigorous not to suffer much in agricultural performance from some slight losses in leaf area (Simmonds, 1948*a*).

It is worthwhile to draw together here various results on leaf characters in relation to ploidy (Simmonds (1948*b*) and the present paper). Table IV shows that, from diploidy to tetraploidy, thickness and weight of leaf and fresh dry weights of petiole all increase. This increase in bulk, however, is not

matched by an equivalent increase in mechanical strength and, as the percentage dry weight of the petiole declines, so also do the rigidity of the leaf and strength of the petiole. Obviously none of these effects is simple and, indeed, whenever the data has permitted a suitable analysis, it has been evident that there are complicated genetical and developmental interactions.

TABLE IV

Summary of the Effects of Ploidy upon the leaf of Musa

	Ploidy		
	$2x$	$3x$	$4x$
Thickness (μ)	337	393	413
Weight (g.)	438	467	527
Petiole: Fresh weight (g.)	50·1	70·2	77·5
Dry weight (g.)	6·91	8·66	9·58
Dry weight %	13·9	12·3	12·4
Posture	+0·554	+0·173	−0·043
Strength (kg.)	13·4	9·61	8·25

LITERATURE CITED

DODDS, K. S., and SIMMONDS, N. W., 1948: Genetical and Cytological Studies of *Musa*, IX. The Origin of an Edible Diploid and the Significance of Interspecific Hybridization in the Banana Complex. Journ. Genet., xlviii. 285–96.

SIMMONDS, N. W., 1948*a*: Genetical and Cytological Studies of *Musa*, X. Stomatal Size and Plant Vigour in Relation to Polyploidy. Ibid., xlix. 57–68.

—— 1948*b*: The Effects of Ploidy upon the Leaf of *Musa*. Ann. Bot. Lond., N.S., xii. 441–53.

SKUTCH, A. F., 1927: Anatomy of the Leaf of Banana, *Musa sapientum* L., var. hort. Gros Michel. Bot. Gaz., lxxxiv. 337–91.

Acid Metabolism and Respiration in Succulent Compositae

III. Further Experiments with *Kleinia radicans* Haw.[1]

BY

BARBARA M. GOWDRIDGE[2]

AND

D. THODAY

(From the University College of North Wales, Bangor

With nineteen Figures in the Text

ABSTRACT

Evidence has been obtained that starving leaves of *Kleinia radicans* in the summer condition produce a volatile substance, which reacts with potassium permanganate, and which accumulates and poisons the leaves when they are enclosed, as in a Dixon respirometer. A microrespirometer has been designed for use with continuous air-currents. Using this, the course of oxygen intake by leaves in darkness resembles that of CO_2 output (previous results) and shows an initial phase of diurnal fluctuation of O_2 intake. Humidity has also some effect, for an early increase in O_2 intake occurs under dry conditions.

[1] This paper incorporates in part the substance of a thesis (Gowdridge, 1949) accepted for the degree of Ph.D. in the University of Wales.

[2] Present address: Botany School, Cambridge.

[Annals of Botany, N.S. Vol. XVI, No. 63, July, 1952.]

Introduction

THE previous paper of this series (Thoday and Richards, 1944) dealt with the respiration of detached leaves of *Kleinia radicans* during starvation in continuous darkness. The results obtained for oxygen intake were unexpectedly different from those obtained for carbon-dioxide output in another form of apparatus. This finding was the starting-point of the present work.

The curves of CO_2 output had shown in summer a diurnal periodicity, lasting sometimes for 2 days, which was very closely parallel to that observed by Bennet-Clark with leaves of *Crassula lactea* (1933). Later in the year this

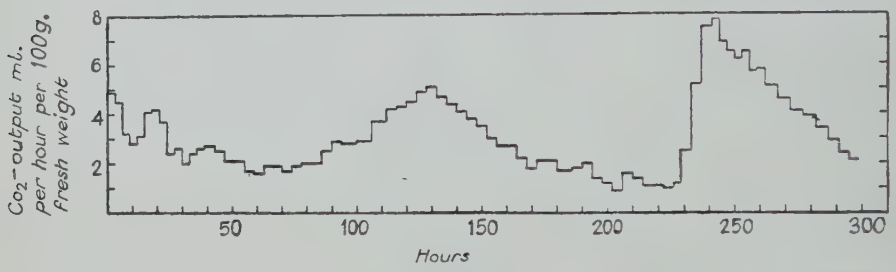


FIG. 1. Thoday and Richards (1944), p. 195, Fig. 11*a*.

periodicity was no longer shown and leaves succumbed to starvation progressively sooner as the season advanced. The diurnal fluctuation was, however, again obtained after exposing plants to the light of a 500-watt Mazda lamp for 12 hours daily for a few days. Leaves from such plants gave a curve of CO_2 output showing three phases (Fig. 1). After a first phase of diurnal fluctuations the curve rose from a low level to a broad maximum extending over some 4 days. The third phase was marked by a sharp transient rise in CO_2 production. In particular cases the peak was reached within 10 hours and was two or three times as high as the maximum rate in the previous phase. The rate had fallen again sometimes within 30 to 40 hours of its rise. The leaves when finally examined were found to have their air-space system completely injected with sap, but had not turned yellow. No parallel has been found for this burst of CO_2 output in starvation curves recorded for other leaves by other workers.

While the results of CO_2 output as a whole formed a coherent picture, individual variations provided ample justification for the practice adopted of using a single leaf for each experiment. On the other hand, adjacent leaves from the same shoot gave almost identical curves, and thus it was possible to make parallel observations of CO_2 output and O_2 intake, using two different pieces of apparatus. A modification of the Pettenkofer technique was used for CO_2 output and a manometric apparatus (Dixon's modification of the Barcroft respirometer) for O_2 intake.

In view of the results obtained by Bennet-Clark (1932*b*) for *Sedum praealtum* some divergence of the two respiration curves, during the initial phase,

was to be expected; in fact, however, the O_2 intake rose within 2 or 3 days to a rate out of all proportion to that of the CO_2 output. The leaves in the O_2-intake apparatus also succumbed prematurely. Their injection and death frequently occurred within 5 days, whereas in the other apparatus leaves remained uninjected for at least twice as long. It was evident that the conditions of the Dixon apparatus had an adverse effect upon the leaves enclosed in it.

When respiration measurements were made alternately in the two pieces of apparatus, transferring leaves from one to the other, it was shown that CO_2 output, as well as O_2 intake, was affected by the conditions of the Dixon apparatus. By determining the O_2 intake after varying periods in the CO_2-output apparatus it was shown that the O_2 intake and the CO_2 output follow similar courses through phases 2 and 3; but this method was not applicable to the first phase.

As manometric methods are simple and convenient it appeared desirable, before abandoning them, to investigate the injurious influence and to try to modify the technique so as to obviate it.

At the outset of the present work, following some preliminary experiments of Thoday and Richards (1944), attention was directed to the nature of the deleterious influence and the possibility of counteracting it in the Dixon apparatus itself as ordinarily used (see Section I).

In a second section of the work a modified manometric technique was developed in which the conditions in the leaf-chamber approached those of the Pettenkofer apparatus. The outstanding difference between the Pettenkofer and the Dixon techniques is that in the former an air-current flowed continuously through the leaf-chamber, whereas in the Dixon respirometer the air remained unchanged during observations. The introduction of an air-current in the oxygen technique was therefore attempted and curves for O_2 intake were eventually obtained which corresponded very satisfactorily with the previous CO_2-output curves in form and duration (see Section II).

Section I. The Nature of the Detrimental Influence in the Dixon Apparatus and Attempts to Remove it

Since the preliminary attempts of Thoday and Richards to discover the nature of the deleterious influence had thrown little light upon the problem, differences in the conditions in the two forms of apparatus that might affect the leaves were brought again under review. Differences could occur in the concentration of carbon dioxide or of oxygen, or in the humidity of the air, and the apparatus itself as well as the leaves might be a source of toxic impurities. Though some of the differences were unlikely to affect the respiration appreciably, still less to be directly injurious, they will be considered briefly in turn.

1. *A difference in CO_2 concentration in the air surrounding the leaves*

The air-current in the CO_2 apparatus passed through 50 per cent. potash and dilute baryta before entering the leaf-chamber, but, as there was no

absorbent in the leaf-chamber itself, the air around the leaf contained a proportion of CO_2 which depended on the relative rates of respiration and air-flow. It is estimated that the concentration of CO_2 in the air-current was of the order of 0·03 per cent. In the Dixon respirometer the air was in contact with a large surface of 10 per cent. potash in the bottom of the flask, so that the CO_2 content of the air around the leaf must have been very low. The difference would be too small to have any direct effect on the respiration, but in view of Heath's discovery (Heath, 1950) that some stomata are sensitive to very small differences in CO_2 concentration, the stomata of leaves in the Dixon apparatus may have been more widely open.

2. *A difference in O_2 concentration in the air surrounding the leaves*

In the closed Dixon apparatus the oxygen concentration fell below that in the air-current. In using the apparatus, however, it was necessary to open it to the external atmosphere at intervals to avoid too great a displacement of the manometer as well as to readjust the level of the burette, and generally to leave the taps open overnight. Calculation shows that the oxygen content of the air never fell below about 19 per cent.[1] One experiment was made in the course of the present work in which pure oxygen entered a leaf-chamber to replace that absorbed by the leaves. Compared with a control in which the leaves were continuously enclosed over potash, no significant lengthening of the life of the leaves was observed.

3. *A difference in humidity conditions*

The humidity conditions of the two leaf-chambers were undoubtedly different. In the Dixon respirometer the air was in contact throughout with 10 per cent. potash at 25° C. In the CO_2 apparatus the air-current entered the leaf-chamber, kept at 25° C., after passing through 50 per cent. potash and dilute baryta at room temperature, 7° to 10° C. lower: it must therefore have produced drier conditions around the leaf than those in the Dixon apparatus. More widely open stomata in the latter would be likely to accentuate the difference.

4. *A difference due to toxins in the Dixon apparatus*

(i) *Toxins derived from the apparatus.* In the Dixon respirometer, unlike the CO_2 apparatus, the enclosed air was normally in contact with mercury in the burette and with paraffin in the manometer.

Mercury vapour is known to be toxic to some plant tissues. In an early preliminary test leaves of *K. radicans* were enclosed in a darkened flask with a few drops of mercury. These succumbed significantly earlier than control leaves in a similar flask without mercury. Nevertheless the replacement of the mercury in the burette of a Dixon respirometer by Brodie's fluid[2] had no

[1] The total range of the burette was only 1 ml. out of a total volume of 55 or 60 ml.

[2] An aqueous solution of sodium chloride and sodium tauroglycocholate, with a little thymol.

appreciable effect on the respiration curve or duration of life of a leaf, compared with a control. It was clear therefore that the capillary tubes connecting the burette with the flask suffice to prevent mercury vapour from reaching a toxic concentration, or else that any effect of mercury vapour is masked by that of another factor.

The replacement of paraffin in the manometer by dibutyl-phthalate, a liquid of low volatility, had no recognizable effect on the results obtained and its greater viscosity was a disadvantage in practice.

(ii) *Toxins emanating from the leaves themselves.* Thoday and Richards put forward the hypothesis that the leaves of *K. radicans* produce a volatile substance, perhaps ethylene, which was removed by the air-current in the CO_2 apparatus, but which accumulated and produced the deleterious conditions in the Dixon apparatus. In the latter the air in the leaf-chamber was enclosed, to the extent that any exchange with the outer air could only take place when the taps were open and then only by diffusion through several centimetres of capillary tube. Mass movement of air occurred when the burette-level was lowered, but inwards only.

This comparison of the two forms of respiration apparatus suggested that the volatile toxin and humidity questions would be most likely to repay investigation. The earlier experiments of Thoday and Richards (1944, p. 198) with intermittent air-currents and with charcoal had given some support to the volatile toxin hypothesis. It was however noted that the difference in the humidity conditions was in the reverse direction to that which they had assumed. The negative result they obtained with a current of drier air in the CO_2 apparatus was therefore not directly relevant.

In the experiments now to be described first of all attempts were made to take up the hypothetical toxin, within the Dixon apparatus, by adsorption on charcoal. Then the starvation of the leaves was followed under various humidity conditions. Later on positive evidence was obtained for the production by the leaves of a toxin capable of reducing permanganate.

Experimental Results

I. *Experiments with activated charcoal*

In continuation of the use of charcoal by Thoday and Richards (1944) a quantity of charcoal was introduced into the leaf-chamber of a Dixon apparatus in which starvation respiration was to be followed. Granulated activated charcoal was used as being the most efficient available for adsorption. It was held in a butter muslin container alongside a leaf.

Preliminary blank experiments showed that the charcoal itself produced pressure changes in the respirometer. At first an increase of pressure was observed, which continued for 30 or 40 hours. This doubtless represented an output of adsorbed gases from the charcoal resulting from the raising of its temperature to 25° C. in the water-bath. After this the pressure change was reversed and indicated an intake of

gas by the charcoal, which soon settled to a steady rate, maintained for many days. As this rate was small in relation to the respiratory O_2 intake of a leaf it was possible to make a correction for it from blank readings taken before and after an experiment, care being taken to avoid cooling the charcoal when introducing and removing the leaf and so upsetting the steady state.

Lowry and Hulett (1920) have shown that charcoal in contact with oxygen only comes to equilibrium after months have elapsed, the exact time varying with the character of the charcoal. Unlike adsorbed oxygen, oxygen fixed during this lengthy process could not be recovered as oxygen when the sample was heated, but only as oxides of carbon.

In some experiments carried out in this way, curves of O_2 intake were obtained of the same general form as without charcoal—no evidence was obtained that its introduction was beneficial.

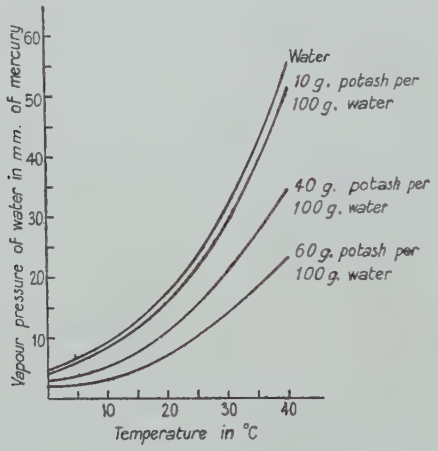


FIG. 2. Graph constructed from data taken from the International Critical Tables (1926).

As the possibility of the toxin being an unsaturated hydrocarbon such as ethylene was envisaged, brominated charcoal was also tried; the results were again negative.

II. *Experiments on the effect of the humidity factor*

1. *The effect of drier conditions in the Dixon apparatus.* It is possible to obtain a rough estimate of the concentration of potash to be used instead of 10 per cent. potash in the Dixon apparatus to produce humidity conditions comparable to those of the CO_2 apparatus. The air-current in the CO_2 apparatus entered the leaf-chamber maintained at 25° C. after passing through, first, a 50 per cent. solution of potash and, second, a dilute solution of baryta, both at room temperature. The concentration of water-vapour must therefore have been less than that represented by the vapour pressure of a dilute solution of baryta at, say, 18° C. This will be seen from the graph in Fig. 2 to be less than 15·5 mm. of mercury—the value for water at 18° C. In the Dixon

apparatus the vapour pressure in the air in equilibrium with 10 per cent. potash at 25° C. would be just over 20 mm. To reduce this to 15 mm. an increase of concentration to rather more than 40 g. potash per 100 g. water (about 30 per cent. by volume) would be required. If the air temperature were less than 18° C., the concentration needed would be correspondingly higher. It was considered unprofitable to attempt to obtain a closer approximation to the concentration of potash required because the extent to which the leaves themselves influenced the humidity conditions in the two pieces of apparatus

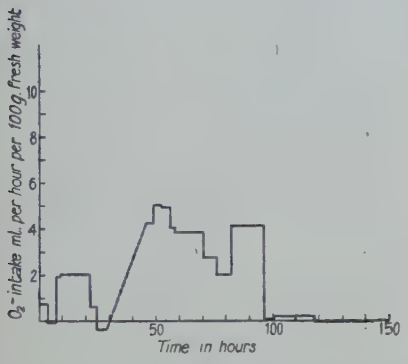


FIG. 3

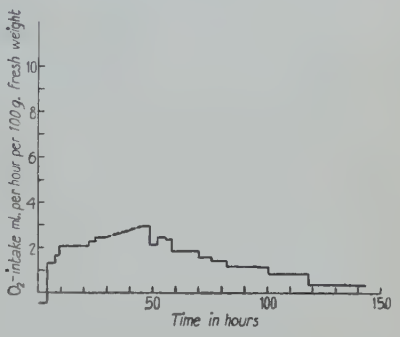


FIG. 4

FIGS. 3 and 4. Expt. IX, started February 12, 1948; leaves darkened at midday. FIG. 3. Carbosorb in flasks; initial weight of leaf, 0·777 g.; leaf examined at 75 hours, almost completely desiccated. FIG. 4. 10% KOH in flasks; initial weight, 0·587 g.; leaf examined at 75 hours, not yet injected.

was unknown. In the one case the air around the leaf was continually replaced, in the other any movement of water-vapour from the neighbourhood of the leaf was mainly by diffusion.

In the first experiment the effect of a very dry atmosphere in the Dixon apparatus was investigated. Comparable leaves were enclosed in two respirometers, one as control over 10 per cent. potash, the other over 'carbosorb', a soda-asbestos preparation, approximately 63 per cent. Na_2O, which besides absorbing CO_2 is a dehydrating agent. The respiration curves obtained were very different (Figs. 3 and 4). After 75 hours the leaf over carbosorb was almost completely desiccated, while the control leaf showed little change. The drying effects of the carbosorb had therefore been drastic, but it was clear that at least extreme conditions of humidity affected the course of starvation in the Dixon apparatus.

In the next experiment a third respirometer was added, containing 50 per cent. potash. All three curves differed from one another (Figs. 5, 6, and 7). Examined after 140 hours the leaf over 10 per cent. potash was completely injected and purple in colour, that over carbosorb completely desiccated. The leaf over 50 per cent. potash, on the other hand, was still quite healthy in appearance, though it showed signs of drying in a wrinkling of the surface, and

it was still respiring vigorously; thereafter the rate of O_2 intake fell, reaching a low level after 200 hours, and when the leaf was examined after 250 hours it was completely desiccated.

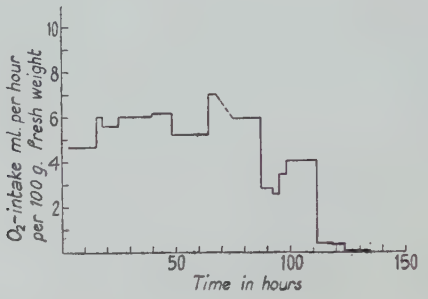


FIG. 5

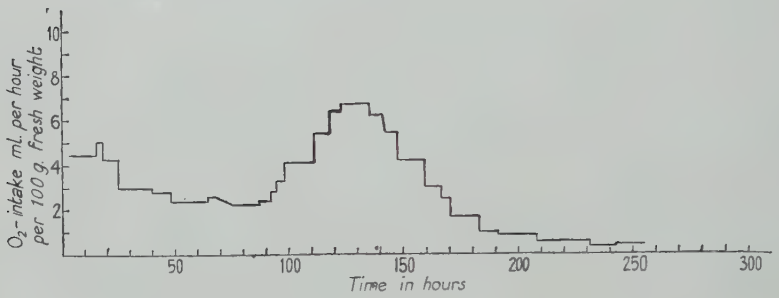


FIG. 6

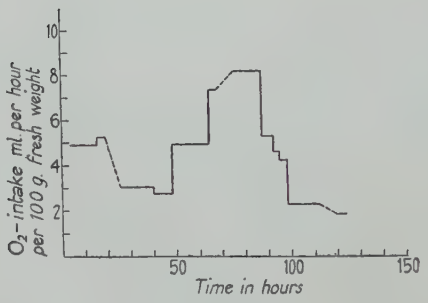


FIG. 7

FIGS. 5–7. Expt. X, started April 30, 1948; leaves darkened at 7 p.m. FIG. 5. Carbosorb; initial weight, 1·002 g.; desiccated at 140 hours. FIG. 6. 50% KOH; initial weight, 0·994 g.; still looked healthy at 140 hours, desiccated by 250 hours. FIG. 7. 10% KOH; initial weight, 1·072 g.; by 140 hours completely injected, purplish.

This experiment showed that a sufficiently dry atmosphere might prolong considerably the life of a leaf. As, however, leaves in the CO_2 apparatus had not become desiccated, the air over 50 per cent. potash was still too dry and it was necessary to try concentrations intermediate between 50 and 10 per cent.

The concentrations selected were 25 and 40 per cent., with 10 per cent. again as a control. A mishap necessitated the restarting of the 25 per cent. respirometer with a fresh leaf 2 days late; but after 92 hours in this case and 140 hours in the other two it was clear that no prolongation of life had occurred with either 40 per cent. or 25 per cent. and the O_2-intake curves were very much

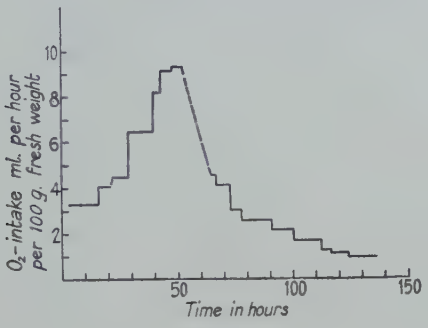


FIG. 8

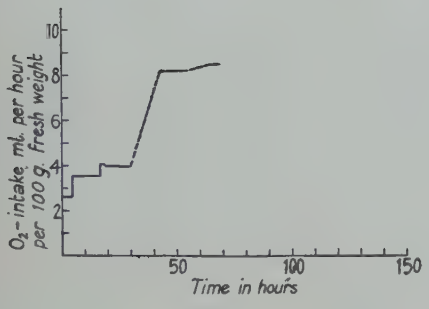


FIG. 9

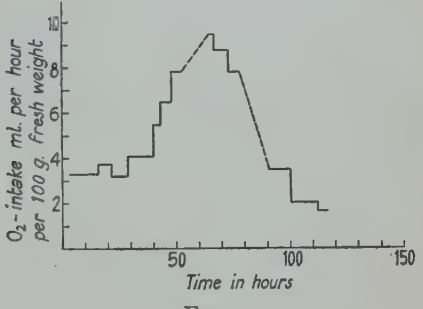


FIG. 10

FIGS. 8–10. Expt. XI, started May 19, 1948; leaves darkened at 6 p.m. (except apparatus with 25% potash (Fig. 9) started May 21, 1948; leaf darkened *c.* 6 p.m.); leaves from same shoot as in expt. X. FIG. 8. 40% KOH; initial weight, 1·202 g.; at 140 hours extensive injection, but also signs of drying. FIG. 9. 25% KOH; initial weight, 1·174 g.; at 92 hours extensive injection. FIG. 10. 10% KOH; initial weight, 1·086 g.; at 140 hours completely injected.

alike (Figs. 8, 9 and 10). The leaf over 10 per cent. potash was completely injected, that over 40 per cent. showed an extensive patch of injection but some signs of drying, while that over 25 per cent. was already becoming injected. After this experiment it seemed unlikely that humidity had been the major factor differentiating the Dixon respirometer from the CO_2 apparatus. The surprising difference between the results with 40 and 50 per cent. potash remains unexplained.

While detailed comparison of the respiration curves obtained in the experiments would not be profitable, some comments on them may be offered. The controls in the three experiments are sufficiently different to emphasize the

need for such controls. The difference between the low level of O_2 intake of the control leaf in the first experiment and the higher level in the other two is no doubt an example of a seasonal change in the condition of the leaves. The first experiment was carried out in February, the other two in the first and second halves of May. In the two latter experiments all the leaves were taken from the same shoot, basipetally in succession. Differences in details between the control curves here probably reflect differences in the external conditions —hours of sunshine, temperature, watering—to which the plants were exposed in the period immediately preceding the particular experiment, with possibly some effect of age and position of the leaves on the shoot.

The evident effect of drying on the respiration of leaves in the experiments just described led to a preliminary investigation of the effect of initial differences in water content before darkening. Significant differences were not obtained, but it was discovered incidentally that leaves with their cut ends in water are unable to make good their transpiration losses by absorption through the cut end. The leaves of *K. radicans* have a longitudinal groove on the adaxial surface beneath which the chlorenchyma is interrupted and water tissue approaches the surface. When short of water the leaves fold in at the groove, the process being reversible in leaves on the plant. A leaf which showed by the closure of the groove that it had incurred a water deficit was placed with its cut end in water, with the object of increasing its water content in relation to an adjacent leaf from the same shoot. However, although, it was covered with a bell-jar to maintain a moist atmosphere, the groove was not observed to expand except in that small part of the leaf which was actually submerged. The blocking of the cut end by oil escaping from cut oil-ducts seems to afford only a partial explanation of this observation. The water relations of these leaves might therefore repay investigation.

2. *The effect of a more humid air-current.* A preliminary attempt was made to reproduce the humidity conditions of the Dixon apparatus in a simple air-current system. In an experiment started on August 12, 1948, leaves were held in darkened boiling-tubes over 10 per cent. potash (*a*) in stagnant air, as controls; (*b*) with a slow current of air humidified by passage through 10 per cent. potash, submerged along with the leaf-chambers in the water-bath at 25° C. Their starvation was followed by inspection only. The leaves in the control tube reached the final injection stage within 4 days, those with a humid air-current did not show signs of reaching a similar condition till after 8 or 9 days. Although no doubt the air-stream had not reached the full degree of humidity corresponding to equilibrium with the potash solution, this result may be taken as indicating the beneficial effect even of a moist air-current.

III. *Evidence that a volatile organic substance is produced by the leaves of* Kleinia radicans *in the dark*

In the last of the experiments described in connexion with the effect of humidity, as an observation incidental to the main experiment, the air-current was taken through a wash-bottle containing a dilute solution of potassium

permanganate after it had passed over the leaves. It was hoped that any volatile toxin produced by the leaves might be capable of reducing the permanganate and be present in sufficient quantity to bring about its decolorization. Unsaturated hydrocarbons as well as other organic substances reduce permanganate.

Decolorization was obtained in the first experiment and it was fairly soon confirmed that a reducing substance was produced in the last injection stage of starvation. Elaborate precautions, however, had to be taken before reliable evidence of its production in the earlier stages could be obtained. The following were among them:

1. Before it was taken over the leaves the air-current was passed through a wash-bottle containing a strong solution of potassium permanganate in order to remove reducing substances present in the laboratory air. It was then passed through a dilute solution to confirm their removal.

2. Careful preparation of the permanganate solutions used was necessary because of their tendency to decolorize spontaneously. They were prepared from Analar crystals using freshly boiled distilled water and were carefully filtered (Cumming and Kay, p. 101). The solutions were kept in the dark throughout.

3. Extreme care had to be taken to avoid the reduction of the permanganate by substances originating from some part of the apparatus other than the leaves. Contact of the solutions with rubber was especially undesirable and it was used as little as possible in the apparatus. Every part of the apparatus had to be scrupulously clean and silicone grease was used in preference to vaseline on ground-glass joints. Finally, as far as possible, parallel control experiments without leaves were carried out.

4. Latterly the special micro-absorption tubes, previously used by Thoday and Richards for baryta in the CO_2 apparatus, were used, instead of wash-bottles, for the permanganate solutions in order to give improved absorption. Three such tubes were set up in series after the leaf-chamber.

After a series of experiments in which this technique was developed, two were carried out which provided evidence for the production of a reducing substance by the leaves throughout the starvation period. The three absorption tubes after the leaf-chamber showed decolorization of the permanganate solution in order, the first before the second and the second before the third, as starvation proceeded. In the second of these two experiments there was an interesting indication, from the rates of decolorization of the three absorption tubes, that the rate of production of the reducing substance may be higher at the beginning and end of the starvation period than it is in the intervening period. This, however, needs confirmation.

In a different type of experiment it was confirmed that the toxin itself is removed by permanganate. Air was circulated within a closed system: it was withdrawn from a vessel containing starving leaves, taken through a wash-bottle containing permanganate and back again to the leaf-chamber, by means of a pump similar in principle to that described by Bennet-Clark (1932*a*).

As in the Dixon apparatus, 10 per cent. potash was present in the leaf-chamber.

Leaves in this apparatus were compared with others in a control experiment in which water was used instead of permanganate. Closely comparable material was used: each apparatus contained three leaves from three pairs of adjacent leaves. The same pump controlled the circulation in the two pieces of apparatus. In the control experiment the leaves were completely injected and olive-brown by the eighth day, while those with air circulating through permanganate were still a healthy green.

In a later repetition of the experiment under improved conditions, seven successive leaves from one shoot were used, the four odd numbers for the control set, the other three with permanganate. The control set were all extensively injected by the fifth day; the others were still a healthy green on the tenth day, two were injected by the twelfth day, but the third was still unchanged in appearance.

There is no doubt that in these experiments the leaves produced a toxin, which was removed by the permanganate and not by passage through water. It is also important that the permanganate produced an improvement in the conditions of a closed system essentially similar to that of the Dixon respirometer without appreciably affecting the humidity conditions. It should be added that these experiments were carried out in August (August 1948); it remains a possibility that there is a seasonal variation in the amount of toxin produced.

Preliminary attempts were made to identify the toxin. Two tests for the presence of an aldehyde were tried. In one air was taken from over a large quantity of material and passed through dimedone—no precipitate was obtained. The second was on a smaller scale using 2:4-dinitrophenylhydrazine which again gave no precipitate. A preliminary attempt to use the pea-test for traces of ethylene also gave no indication of its presence. These negative results cannot, however, be regarded as conclusive.

Section II. The Use of an Air-current in an Apparatus for Measuring Oxygen Intake

In one of their preliminary experiments using a Dixon respirometer Thoday and Richards drew an air-current over three leaves during the intervals between readings of oxygen intake, but they obtained no evidence that this produced a significant improvement in the conditions in the apparatus. A single reading involved closure of the respirometer for at least 2 hours; apparently interruption of the air-current for such long periods was sufficient to invalidate any beneficial effect of it. A 2-hour reading was necessary partly because of the limited sensitivity of the manometer of a Dixon apparatus and partly because of errors inherent in the method, chief among which is the error due to air-temperature changes affecting that part of the apparatus not submerged in a water-bath.

A manometric apparatus has therefore been constructed which is more

sensitive than the Dixon apparatus and free from its main source of error—when in use it is wholly submerged in a constant-temperature water-bath. With it a reading involves interruption of the air-current for only 20 minutes.

The development of this form of apparatus presented some technical difficulty, but its completion was regarded as a matter of first importance,

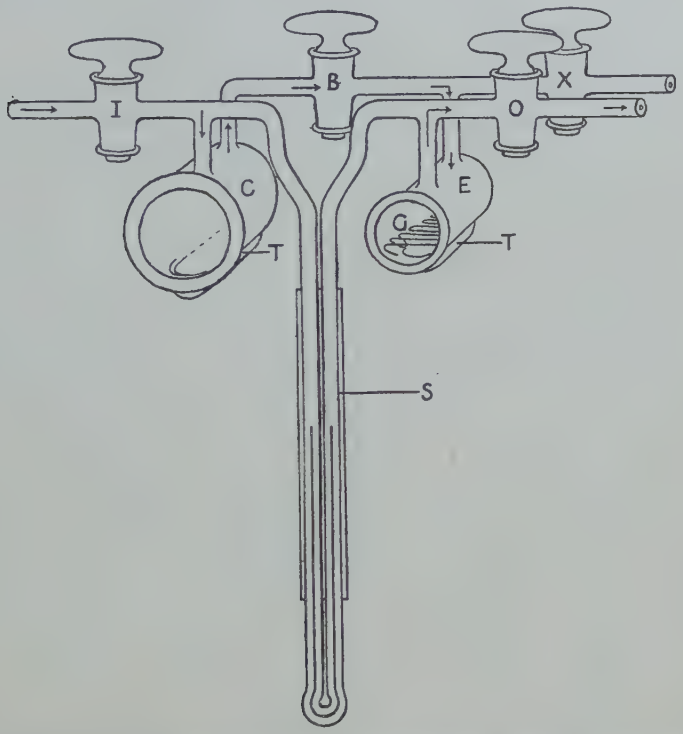


FIG. 11. Diagram of microrespirometer: E, experimental vessel; C, compensating vessel (the cover-plates of C and E are not shown); T, troughs for CO_2 absorbent; G, platinum grid for leaf; I, O, B, X, inlet, outlet, by-pass, and calibration taps; S, mirror scale attached behind manometer. Length of chambers (from back to front) *c.* 6 cm. (see text).

because the use of an almost continuous air-current in an oxygen-intake apparatus was expected to produce conditions approaching those of the carbon dioxide apparatus, whatever their nature.

1. *The microrespirometer*

The final form of apparatus which was brought into use is shown in Fig. 11. It consists essentially of a manometer connecting two glass vessels, the smaller of which is the experimental vessel and the larger the compensating one. In both the lower side is expanded into a trough for holding a carbon-dioxide absorbent, usually a 10 per cent. solution of potash, and, in the experimental vessel, a detached leaf was supported above it on a platinum grid. Thus with all the stopcocks closed the oxygen intake of such a leaf, when darkened,

would appear as a displacement of the manometric fluid towards the experimental vessel.

At the front each vessel is moulded so as to present a larger area of ground surface to the flat ground-glass plate with which it is covered. It was found desirable to use unwettable silicone grease on these joints as on the stopcocks, and when setting up the apparatus the cover-plates were eased on to the vessels until a firm glass-to-glass contact was felt. As an added precaution the plates were then held in position by a simple clamp.

A mirror centimetre scale is mounted behind the manometer. As it was particularly important that the leaves should not be exposed to any toxic vapour the paraffin used in the manometer was purified and redistilled. It was coloured with one of the Sudan dyes. The manometer was thoroughly cleaned before introducing the indicator fluid.

When in use the respirometer is submerged in a constant-temperature water-bath. Accurate control of temperature was found to be necessary; it did not vary by more than 0·03° C. from part to part of the bath used, which was maintained at a temperature of 25° C. This was achieved by using a proportioning head, supplied by Sunvic Controls Ltd., attached to the mercury-toluol thermoregulator, by vigorous stirring and by reducing the framework supporting the apparatus to a minimum. Leaves in the apparatus were darkened by a system of curtains arranged outside the water-bath.

In the experiments which follow, an air-current taking the course shown by the arrows in Fig. 11 was passed through the apparatus continuously except for infrequent interruptions (at first only two per day) each of 20 minutes or half an hour. After leaving an aspirator the air was warmed, by passage through a 150-cm. length of 4-mm. bore copper tubing immersed in the water-bath, before entering the apparatus. The rate of flow of the air-current was of the order of 150 ml./hr.

When the air-current was interrupted in order to take a reading of oxygen intake the procedure was as follows: The current was first stopped outside the water-bath and the respirometer was left for 10 minutes with the stopcocks open to allow for equilibration of conditions on either side of the manometer. The stopcocks were then closed in turn, the by-pass stopcock B last bringing the manometer into action. At the moment of closing the by-pass a first reading was made of the position of the fluid in one arm of the manometer, observing it through the glass front of the water-bath. This was repeated after 10 minutes. Occasionally a second measurement of oxygen intake was made over the next 10-minute period before starting the air-current again; the agreement between such consecutive measurements, examples of which can be seen in Fig. 14, indicates the degree of accuracy of the results.

The principle of the respirometer when it is used in this way is that of a Barcroft differential manometer. The calculation from the readings of the manometer of the absolute volumes of oxygen intake involves a factor for the volume of the respiring material which is difficult to estimate in the case of a leaf and this was not attempted. The readings were roughly calibrated using

some results obtained relating movement of the manometric fluid to that of liquid in a burette temporarily attached to the experimental vessel. In the present experiments a knowledge of the general trend of oxygen intake over a long period was more important than the accurate calculation of the absolute values and this rough calibration was sufficient.

Measurable displacement of the manometric fluid occurs in response to volume changes in the experimental vessel of as little as 0·25 mm^3. This represents a sensitivity four times that of the Dixon apparatus. Moreover, the error which limits the performance of the microrespirometer is that involved in reading the manometer, whereas in the Dixon apparatus there may be in addition an error due to air-temperature changes of at least the same order of magnitude.

Although so far it has been convenient to use this apparatus as a respirometer of the Barcroft type, more accurate measurement of absolute volume changes would be achieved using it as a Dixon respirometer. A burette would be permanently attached to the branch x provided for this purpose and used to level the manometer. Readings of the burette would give volume changes directly. Further, the sensitivity of the apparatus could be increased by observing the levelling of the manometer through a horizontal microscope.

It may be useful to record some details of the way in which this final form of apparatus was reached and some of the difficulties arising in its use.

The first apparatus to be assembled incorporated some of the features of a microrespirometer due to Stefanelli (1937), an apparatus which is similar to the Dixon respirometer in principle but more sensitive because of its reduced dimensions. Later, a second drastically modified apparatus was constructed, but it was only with a third final form that success was achieved. The method of closing the experimental and compensating chambers described by Stefanelli was retained in the third apparatus—flat glass plates were thought to be preferable to ordinary ground-glass joints which Thoday and Jones (Richards) (1939) had found troublesome in the Dixon apparatus because of minor movements of one part of the joint on the other. Similar considerations led to the use of mercury taps resembling those of Stefanelli's respirometer, instead of stopcocks, in the first apparatus. They were not retained in the later forms of the apparatus because it seemed desirable to eliminate all traces of mercury, with the possibility of its vapour poisoning the leaves. No evidence was obtained that stopcocks were unsatisfactory. It may be noted that the stopcocks occasionally became blocked and had to be cleaned with a piece of wire; access may be had to the by-pass stopcock through the tap x.

The very accurate control of temperature already described was introduced following the observation that when an ordinary mercury-toluol thermoregulator was used the rhythmic rise and fall of temperature in the water-bath brought about minor movements of the manometric fluid. No further fluctuation of this sort occurred.

Throughout the development of this apparatus the most serious difficulties arose in connexion with the use of the air-current. In blank experiments pressure changes occurred in the respirometer after the air-current had been stopped; they were generally sufficient to interfere with a reading, lasting for at least 10 minutes and being of the same order of magnitude as the pressure change brought about by a leaf in a 10-minute period.

A great deal of time was spent on attempts to identify and eliminate the factor or factors responsible for this after-effect of the air-current. Some of the more obvious possibilities were examined with the first apparatus. The use of copper tubing was introduced so that the air-current was brought to the temperature of the water-bath before entering the apparatus, and a first attempt was made to adjust the humidity of the air-current to correspond with that in the respirometer. It was demonstrated that the after-effect of the air-current was not associated with mechanical movement at the ground-glass joints between the cover-plates and the chambers of the respirometer. Even when a plate was moved appreciably by hand any resulting pressure change was very much smaller than those under consideration.

It was disconcerting that consistent results were never obtained in blank experiments using the first apparatus. One series of measurements of the after-effect—alike among themselves—would be followed, for no apparent reason, by another series differing not only in the amount but also in the direction of the pressure changes recorded. Finally it could only be supposed that leakage of minute amounts of water into the apparatus, perhaps even through the vaseline of the ground-glass joints, was responsible.

In this respect the second apparatus was an improvement upon the first because it was made entirely of glass, also unwettable silicone grease was used instead of vaseline on all ground-glass joints. However, because of a faulty stopcock, the possibility of leakage could not be wholly eliminated until the third apparatus was brought into use.

Another modification introduced with the second apparatus was in the course of the air-current. Whereas in the first apparatus it had passed through the experimental chamber only, it was now taken through both chambers and they were made as alike as possible so as to minimize any differential effect produced by the air-current. The third apparatus resembled the second in this respect but the size of the compensating chamber was increased to give improved sensitivity: no evidence was obtained that this was disadvantageous.

The effect of the air-current in the third apparatus was consistent and the results obtained with it are worth examining in more detail. Using the respirometer quite dry and empty, an unmoistened air-current, warmed by passing through a short length of copper tubing, produced no significant after-effect. On the other hand, when 10 per cent. potash was introduced into the chambers after-effects were at once observed; with the air-current entering the compensating chamber first, the manometer indicated an increase of pressure on the compensating side after the stopping of the current. The movement was virtually complete within 10 minutes, provided that the current had not been

unnecessarily fast, but it was still comparable in magnitude with the movement that would be produced by the oxygen intake of a leaf in the same time.

The movement could be explained by supposing that the relatively dry air-current picked up water-vapour in passing over the potash in each chamber in succession, so that when the air-current was stopped a greater residual moisture deficit would remain in the air in the first chamber. In the subsequent process of equilibration with the potash the pressure would therefore be raised in the first chamber more than in the second.

In an unsuccessful attempt to eliminate the effect, the air-current was passed, before it entered the apparatus, through an elaborate type of wash-bottle containing 10 per cent. potash, immersed in the water-bath at 25° C.; the significance of this negative result is however in doubt because the rate of humidification may well have been too slow in the wash-bottle, in spite of the good contact between air and solution.

On the other hand, when the air for the current was drawn from a reservoir of air submerged in the bath, in contact with the water in the bath, the air being replaced by water from the bath as it was withdrawn, an after-effect was obtained in the same direction as before. It remained uncertain therefore whether humidity changes could wholly account for the after-effects observed.

It is clear, however, from other experiments that humidity is a major factor. In these the potash in the chambers was replaced by carbosorb (p. 355) and the air-current was moistened by passage through the special humidifier. In this case equilibration after closure would be in the reverse direction, by removal of water-vapour; the pressure changes would be negative, but again greater in the control chamber. Accordingly a movement in the manometer in the direction opposite to that previously observed was to be expected. It was in fact obtained and was repeatable. Replacement of the carbosorb by calcium chloride, with the same result, appeared to establish conclusively that the major part at least of these after-effects was to be explained in terms of humidity.

By this time it was clear that such after-effects could not be eliminated, particularly as the leaf itself would be a complicating factor affecting humidity. Since, however, the equilibration was practically complete 10 minutes after closure, it was decided to proceed with respiration experiments, allowing 10 minutes to elapse before closing the by-pass, and to determine the O_2 intake during the following 10 minutes.

It should be added that one reference has been found in the literature to the use of an air-current in a similar piece of apparatus, by Schmitt (1933). He also observed volume changes in blank experiments and in spite of various precautions did not succeed in wholly eliminating an 'apparatus drift'.

2. *Experiments with the microrespirometer using an air-current*

The microrespirometer was used successfully during the summer of 1949. Evidence that the conditions in it were an improvement upon those of the Dixon apparatus and were indeed as favourable as those of the previous CO_2

apparatus was provided both by the increased length of life of leaves used in it and by the form of the O_2-intake curves they gave. This is best illustrated by two experiments of which the results are given in Figs. 12 and 13.

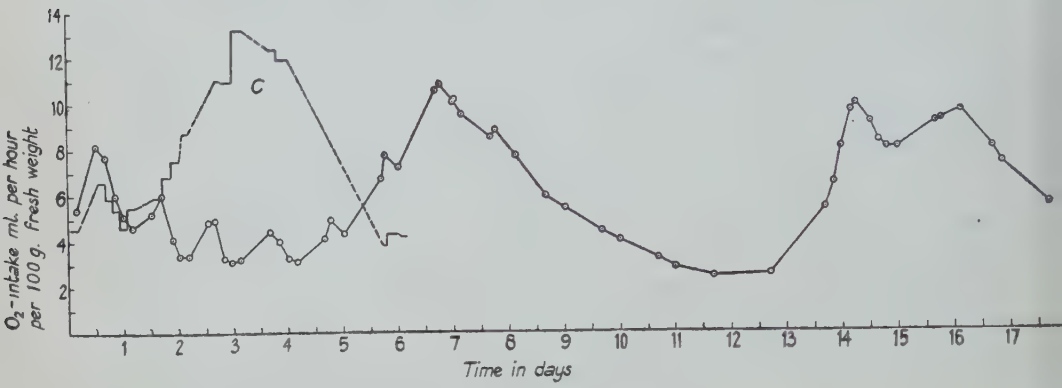


FIG. 12

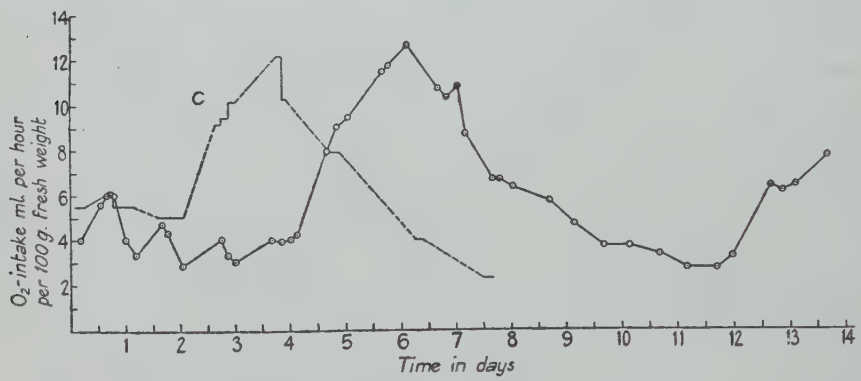


FIG. 13

FIG. 12. Expt. XXV, started July 25, 1949, using microrespirometer with 5% KOH in chambers; C, control in Dixon apparatus: both leaves darkened *c.* 6 p.m.; initial weights, 0·582 g. and (control) 0·566 g. Control injected internally and partly brown after 6 days; first definite signs of collapse in the other leaf about the 14th day. FIG. 13. Expt. XXVI, started August 12, 1949, as expt. XXV, but with humidifier containing 10% KOH, immersed in bath, inserted in air circuit before the microrespirometer; leaves darkened 6 p.m.; initial weights, 0·449 g. and (control) 0·537 g. Control leaf olive-brown by 8th day, in parts earlier; other leaf showing first signs of collapse about the 11th day, discoloration becoming general on the 12th day. *Note.* In Figs. 12 and 13 the time scale is given in days instead of hours to indicate the diurnal periodicity of O_2 intake. The magnification is however the same as that of the other graphs in this paper.

Both these O_2-intake curves resemble previous CO_2-output curves in the three phases of respiration which they show. The first phase of diurnal periodicity of O_2 intake is especially interesting. In the experiment started on July 25 (Fig. 12) the periodicity persisted for 6 days, overlapping the second phase, and there was even a suggestion of it on the eighth day; this is, as far as

we are aware, longer than has previously been recorded for respiration (as CO_2 output) during starvation in this or any other succulent. This makes parallel observations of O_2 intake and CO_2 output in one leaf or in closely comparable leaves highly desirable. Meanwhile comparison with CO_2-output curves previously obtained (Thoday and Richards, 1944, p. 192) suggests that (during the first 2 days) the maxima and minima of CO_2 production do not coincide with those of O_2 intake.

The third phase, though not as prominent as the burst of CO_2 output in some of the summer experiments previously described, is comparable with

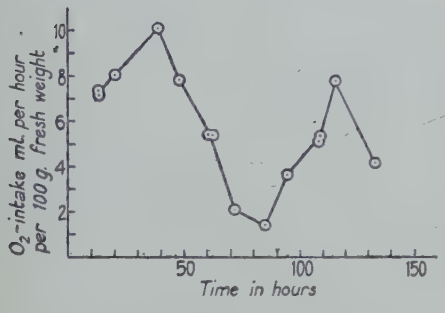


FIG. 14

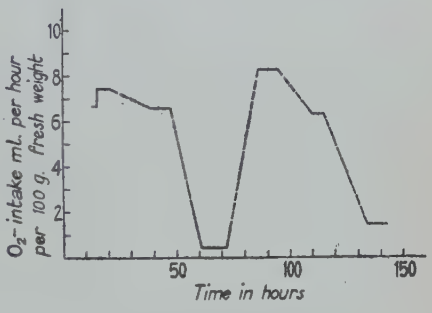


FIG. 15

FIGS. 14 and 15. Expt. XIX, started February 11, 1949; leaves gathered at 6.30 p.m., kept in almost complete darkness until experiment started 9.30 p.m. Leaves had received light treatment (see p. 350) for 8 days. FIG. 14. Microrespirometer, 10% KOH in chambers; initial weight, 0·858 g. Patches of injection after 50 hours; after 75 hours injection extended, but less complete than in control. FIG. 15. Control; initial weight, 0·650 g.; completely injected after 75 hours.

others. (Part of the O_2 intake at the very end was due to mould developing on the injected tissue.)

In contrast with the above experiments the first results obtained with the microrespirometer—in January and February 1949—showed no significant difference from those of parallel control experiments in the Dixon apparatus. This may be illustrated by the experiment shown in Figs. 14 and 15. Why was there no evidence here of improved conditions in the microrespirometer? At the time it seemed possible that the humidity was detrimentally high in the microrespirometer because in these experiments the air-current entered the apparatus after passing through an elaborate humidifier containing 10 per cent. potash at 25° C. (see p. 365). This, however, is unlikely in view of the summer experiment quoted above (Fig. 13), in which the conditions can hardly have been less humid; the humidifier was again used and 5 per cent. instead of 10 per cent. potash was used in the chambers of the apparatus. It now seems much more likely that the contrast between the summer and winter results reflected a difference in the condition of the leaves used, perhaps even a difference of sensitivity to humidity. It may not be merely a question of a seasonal difference in the leaves, or at least not a difference related to prior illumination,

because some of the leaves used in the winter experiments had received light-treatment. The earlier experiments of Thoday and Richards, who obtained a three-phase CO_2 curve for light-treated leaves in January 1937, suggested that light-treatment could induce a condition substantially similar to that of summer leaves. On the other hand, this may not have been true of the leaves used in the winter of 1949, and the similar O_2-intake curves obtained with the microrespirometer and the Dixon apparatus may both have given an accurate picture of their starvation respiration. If so, this is evidence that little or no toxin was produced by these particular leaves; but no experiments with permanganate solutions were carried out in the winter and there is therefore no direct evidence on this point.

It was a constant source of difficulty throughout this work that the condition of the leaves, and correspondingly their starvation respiration, were affected by the amount of sunlight received in the period immediately before they were used and by other factors, some of them not connected with changes of season (p. 358). Among these factors, which have not been studied separately, the age of the leaves and the mineral nutrition they received were probably important. There was some visual evidence that the leaves in the summer of 1949, which was an exceptionally sunny one at Bangor, were in a condition which had not been noted previously. The leaves were tougher and became injected less readily after starvation, or showed it less obviously at the surface. Some yellowing and sometimes considerable browning of the leaves were associated with the later stages of starvation. The leaves of plants that had received supplementary mineral nutrition and were growing more luxuriantly did not show this type of discoloration but became obviously injected while still green.

A series of experiments was carried out in May, June and July, 1949, following the winter experiments and in connexion with the hypothesis that the humidity in the apparatus had been detrimentally high. It was found possible to discard the humidifier and the air-current was passed through 50 per cent. potash and dilute baryta at air temperature before entering the apparatus in order to reproduce the humidity conditions of the air-current in the CO_2 apparatus. It was not accurately known what concentration of potash should be used in the chambers of the apparatus to correspond with this (see p. 355) and different concentrations (50, 40, 30, 20, 10, and 5 per cent.) were tried in turn. (The results for the 5 per cent. potash experiments have already been shown in Figs. 12 and 13. The leaf was shrivelled after only 3 days in the experiment with 50 per cent. potash and it was discontinued. Results for the remainder are given in Figs. 16 to 18.) There was evidence of too rapid drying with 40, 30, and 20 per cent. potash.[1] This series of experiments therefore records the effect of conditions of humidity in the micro-

[1] Drying was more rapid in these experiments than over corresponding solutions in the Dixon apparatus (see pp. 354 et seq.). The conditions in the microrespirometer differed in the movement of the air in the chambers and in the proximity of the potash solution to the leaf.

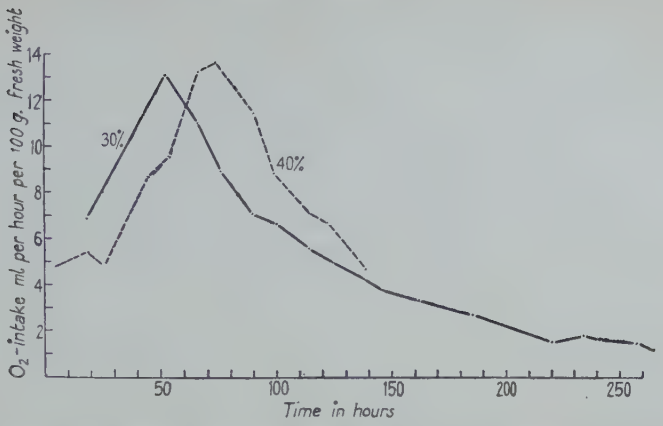


FIG. 16. Expt. XXI, started May 27, 1949; 40% KOH; darkened at 4.18 p.m.; initial weight, 1·053 g.; after 110 hours much shrivelled, but no other sign of deterioration; final weight, 0·233 g. Expt. XXII, started June 3, 1949; 30% KOH; darkened at *c.* 4 p.m.; initial weight, 0·991 g.; some shrinkage by 7th day; by 13th day dry, tip brown.

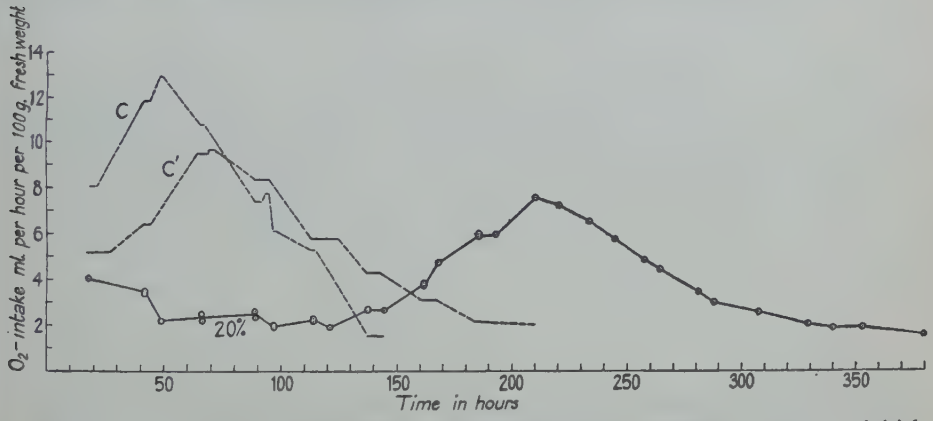


FIG. 17. Expt. XXIII, started June 16, 1949; 20% KOH; darkened *c.* 4.50 p.m.; initial weights, 1·054 g. and (control c) 0·834 g. As first control leaf showed unusual symptoms, the control was repeated with another leaf (c′). (Started June 24; initial weight, 0·890 g.; darkened at 4.15 p.m.) This also showed signs of necrosis by the 6th day and was completely moribund by the 10th day. The leaf in the microrespirometer remained healthy in appearance except for progressive shrinkage until it collapsed and developed mould on the 21st day.

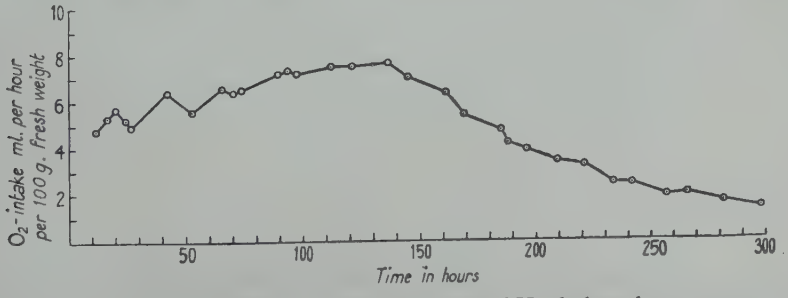


FIG. 18. Expt. XXIV, started July 8, 1949; 10% KOH; darkened at 4.20 p.m.; initial weight, 0·919 g. Partial injection on 15th day, rapidly extending; final weight July 24, 0·677 g.

respirometer ranging from very dry to relatively moist (5 per cent. potash). The early rise of O_2 intake under very dry conditions (with 40 and 30 per cent. potash) agrees with that occurring in the Dixon apparatus when carbosorb was used (p. 356). The curve for 20 per cent. potash is intermediate in duration between those for 30 and 5 per cent. and approaches the latter in form, but the leaf lost 80 per cent. of its initial weight within 3 weeks.

The leaves used in this group of experiments were as alike as possible and were all taken from the same shoot. The leaf used with 10 per cent. potash

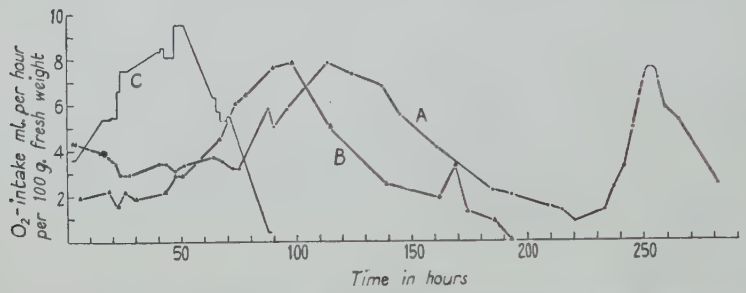


FIG. 19. A, Expt. XXVII, started October 25, 1949; microrespirometer, with air circulated through aqueous permanganate but not renewed (with control, C, in Dixon apparatus); darkened *c.* 6 p.m.; initial weights, 0·508 g. and (control) 0·574 g. (In this experiment the temperature of the bath fell during the first 20 hours (A) owing to the unsuspected failure of an immersion heater: the first two values have been corrected approximately to 25° C.) B, Expt. XXVIII, started November 7, 1949; as expt. XXVII, but air circulating through water only; darkened *c.* 4 p.m.; initial weight, 0·474 g. (For details see text.)

was however exceptional in being older than the rest (farther back from the apex) when it was taken for experiment; this may account for the anomalous form of this curve which breaks the sequence. The similarity of the other leaves justifies the interpretation of the differences between these curves as differences due to the humidity factor, but as the curves form a time sequence the results may be to some extent affected by the seasonal progression.

3. *Oxygen intake from air internally circulated*

It now seemed very probable that the air-current in the microrespirometer removed a toxin from the vicinity of the leaves or at least had done so in the summer experiments. Also in August 1949 evidence was obtained that this toxin could be removed by circulation of the air through permanganate (p. 359). Oxygen intake was therefore investigated with the microrespirometer connected to the circulating pump previously used, through an absorption tube containing a fairly strong aqueous solution of potassium permanganate.

This experiment was started late in October 1949, along with a control in the Dixon respirometer. The results are shown in Fig. 19, A and C. The control leaf succumbed within 4 days. The other leaf survived three times as long. Its curve of O_2 intake shows both the respiration hump of the second

phase and, very clearly, the final burst during the spread of injection. There is very little sign of diurnal fluctuation at the beginning; this feature is however adequately accounted for by the lateness of the season.

A similar experiment carried out immediately afterwards, with distilled water instead of permanganate in the absorption tube, gave unexpectedly a somewhat similar curve of O_2 intake, though of shorter duration (Fig. 19B). The leaf succumbed 4 days earlier and the O_2 intake fell away rapidly after reaching the first maximum except for a transient rise after 7 days, but the result was of different type from that associated with ordinary controls in the Dixon apparatus. The circulation of air in this case appears therefore to have been beneficial, though less so than when the air had passed through permanganate. As no improvement had resulted in previous experiments from circulation through water, some other explanation seems to be required in this case. The connexions outside the bath between respirometer and circulating pump were unavoidably rather long and rubber tubing had been used for them. If the toxin proves to be a substance to which rubber is permeable this might account for the result obtained in this experiment.

Summary

1. Evidence has been obtained that starving leaves of *Kleinia radicans* in the summer condition produce a volatile substance, which reacts with potassium permanganate, and which accumulates and poisons the leaves when they are enclosed, as in a Dixon respirometer.

2. A microrespirometer has been constructed in which an air-current may be passed over a leaf continuously, except for minor interruptions while measurements of oxygen intake are made. Such leaves in summer in continuous darkness gave extended curves of oxygen intake resembling curves previously obtained for carbon dioxide output; in particular they showed an initial phase of diurnal fluctuation of oxygen intake lasting for several days. These curves provided evidence for the improved conditions of this apparatus as compared with the Dixon respirometer. A similar curve was obtained when the air in the apparatus itself was circulated through an aqueous solution of permanganate.

3. The oxygen intake of the leaves has been followed under various humidity conditions using both a Dixon respirometer and the air-current microrespirometer. The evidence suggests that humidity is not a major factor in the deleterious conditions of a Dixon respirometer. On the other hand, humidity conditions were not without effect: under dry conditions an early increase in the rate of oxygen intake was observed.

Literature Cited

Bennet-Clark, T. A., 1932 (*a*): A Method for automatically recording the Oxygen Intake of Living Tissues. Sci. Proc. Roy. Dublin Soc., xx. 281–91.

—— 1932 (*b*): The Respiratory Quotients of Succulent Plants. Ibid., 293–9.

—— 1933: The Role of Organic Acids in Plant Metabolism, Part II. New Phyt., xxxii. 128.

Cumming, A. C., and Kay, S. A. Quantitative Chemical Analysis. 8th edition 1942. Gurney & Jackson, London and Edinburgh.

Gowdridge, B. M., 1949: On the Effects of Enclosure on the Respiration of the Succulent Leaves of *Kleinia radicans* Haw. and Possible Methods for the Elimination of these Effects. Ph.D. Thesis, University of Wales.

Heath, O. V. S., 1950: Studies in Stomatal Behaviour. V. The Role of Carbon Dioxide in the Light Response of Stomata, Part I. J. Exp. Bot., i. 29–62.

International Critical Tables of Numerical Data, Physics, Chemistry and Technology, Ed. Washburn. 1st edition, 1926. McGraw-Hill Book Co.

Lowry, H. H., and Hulett, G. A., 1920: Studies in the Adsorption by Charcoal. II. Relation of Oxygen to Charcoal. Journ. Amer. Chem. Soc., xlii. 1408.

Schmitt, F. O., 1933: The Oxygen Consumption of Stimulated Nerve. Amer. Journ. Physiol., civ. 303.

Stefanelli, A., 1937: Un nuovo Microrespirometro Manometrico. La Ricerca Scientifica, xv (1). 24.

Thoday, D., and Jones, K. M., 1939: Acid Metabolism and Respiration in Succulent Compositae. I. Malic Acid and Respiration during Starvation in *Kleinia articulata*. Ann. Bot., n.s., iii. 677–98.

—— and Richards, K. M., 1944: Acid Metabolism and Respiration in Succulent Compositae. II. Respiration during Starvation in *Kleinia radicans*. Ibid., viii. 189–203.

Some More Fossil Woods of *Glutoxylon* from South-east Asia

BY

K. A. CHOWDHURY

(*Forest Research Institute, Dehra Dun, India*)

With Plate XX

ABSTRACT

1. Four fossil wood specimens from the Tertiary of South-east Asia are described here. Two are new from India; one is *Dipterocarpoxylon burmense* from Burma and the other *Dipterocarpoxylon annamense* from Indo-China. All of them resemble *Glutoxylon assamicum* and are indistinguishable. They have been placed in the genus *Glutoxylon* and named *G. burmense* (Holden) n. comb.; Holden's specimen having priority is given the specific name.

2. The recent and past distribution of the *Gluta* shows that its centre has shifted about 1,000 miles towards the south-east.

I. INTRODUCTION

THIS paper describes some fossil woods from the Tertiary of South-east Asia. They show affinity with the modern *Gluta*, a genus of commercially important timber trees of the Anacardiaceae. Some of them had been described under several generic and specific names, but it is not possible to distinguish the species from one another. I have, therefore, united all under the one name *Glutoxylon burmense* (Hold.) n. comb.

It is shown that the wood described as *Dipterocarpoxylon burmense* Holden (1919) does not have the anatomical structure of the Dipterocarpaceae but is allied to the *Gluta*. The recent and fossil distribution of the *Gluta* is described and discussed.

II. MATERIAL AND METHOD OF STUDY

The new specimens described in this paper are from two localities. One lot of nearly 20 are from the Cachar Hills of Assam, 25 miles south-east of the locality which yielded the first specimen referred to *Glutoxylon* (Chowdhury, 1936). Their age is said to be Middle Tertiary. They were collected by Mr. P. R. Datta of the Assam Forest Service in 1936. Their preservation is not very satisfactory, but it has been possible to cut fairly good rock sections.

The second specimen comes from a collection of fossil woods made by Mr. A. K. Banerji (Gee, 1932), Geological Survey of India, from the laterite

[Annals of Botany, N.S. Vol. XVI, No. 63, July, 1952.]

formation on the extreme east of Ranigunj coalfield. It bears G.S.I. type No. K24/797. The geological age of the locality is said to be between Miocene and Pleistocene. Banerji's whole collection of specimens is now being investigated by me and the results will be published later.

Of the previously known specimens, there is one reported by Sen (1930) from the Lalmai Hills of Bengal. He referred it to *Dipterocarpoxylon* Holden. Unfortunately the specimen and the slides are not at present traceable and I have only the information given in Sen's paper.

The specimen from the Irrawady series of Burma has a long story attached to it. Two specimens from these series were identified by Holden (1916) as *Dipterocarpoxylon burmense*. In 1934 Bancroft expressed her doubt about the accuracy of this identification. In 1935 Gupta, who had re-examined Holden's original slides and specimens, reported two timbers. One he called *Irrawadioxylon burmense* and the other *Dipterocarpoxylon holdeni*. In 1936 it was pointed out by Chowdhury that *Irrawadioxylon burmense* showed some similarity to *Glutoxylon* and that a re-examination would be necessary before it could be put with certainty in its proper systematic position. Due to the courtesy of Mrs. Sahni, Birbal Sahni Institute of Paleobotany, Lucknow, it has been possible for me to examine the microscope slides of *Irrawadioxylon burmense* prepared by Gupta. The results of the examination are given in this paper.

The specimen from Indo-China was originally reported by Colani (1919) as *Dipterocarpoxylon annamense*. In 1931 Edwards raised some doubts about the accuracy of the identification and remarked that its 'generic identification should be accepted with reserve'.

It does not appear to be necessary to give here a full diagnosis of *Glutoxylon burmense*, because this has been done already (Chowdhury, 1936). For the present it will be sufficient to list its salient characters:

Glutoxylon burmense is a diffuse-porous wood. It has no distinct growth rings. Its vessels are medium-sized to fairly large, single or in radial pairs of 2–4; heavily tylosed, occasionally filled with gum; the perforation plates are simple and nearly horizontal; the intervessel pits are bordered, large, alternate, the border being oval to hexagonal, the aperture lenticular; the vessel-ray pits are 1–5 per ray field, large, narrowly bordered, round to oval. Fibres are moderately thick-walled and arranged in radial rows. Parenchyma cells are paratracheal and apotracheal; the paratracheal cells are in 1–2 rows round the vessel; the apotracheal cells are mostly in bands of 1–2, irregularly spaced, some are continuous, others end abruptly. Rays are simple and fusiform; the simple are uniseriate and the fusiform 3–4 seriate with horizontal gum duct in the centre. Vertical gum ducts are absent.

For further details please see full diagnosis (Chowdhury, 1936).

III. Results of Study

The full description previously published on the specimen referred to as *Glutoxylon assamicum* (Chowdhury, 1936) holds good without any modifica-

tion for the two new specimens from India. The Cachar Hill specimens were not all well preserved, but the best yielded sections which agreed fully in every anatomical detail with the first specimen from Assam. Similarly, the Ranigunj specimen, which is fairly well preserved, agrees fully in every anatomical feature with the first Assam specimen.

The previously described specimens are a little more troublesome because I differ from their authors in the interpretation of some anatomical features present in these woods. They, however, agree fully in anatomy with the Assam specimen and have therefore been identified with it.

Sen reported his specimen from Bengal to be similar to Holden's *Dipterocarpoxylon burmense*. No details are available beyond his note and no conclusion is based on this specimen.

The Burma specimen described by Holden (1916) is represented by slide Nos. 814 and 815 in the Cambridge Botanical School Collection, which I have not seen, but I have studied some sections prepared by Gupta from the same specimen. Two of these slides are figured in this paper (Pl. XX, Nos. 4, 5). The Burma specimen agrees fully in anatomical details with the Assam specimen, and having priority its name is used for the species.

The single specimen from Indo-China was described in some detail by Colani (1919). All its anatomical characters agree with those of the Assam specimen except for the supposed vertical gum ducts, and these are discussed below.

IV. Discussion

As a rule, the anatomical structure of wood at species level is indistinguishable. The botanical genera, however, have well-defined wood structure except in a few cases in which the taxonomically neighbouring genera may show indistinguishable anatomical features. *Gluta* has a good many species with very similar and indeed barely distinguishable wood structure. Another genus *Melanorrhoea* of the Anacardiaceae has more varied structure, some being distinguishable but others just like *Gluta*. The name *Glutoxylon* thus refers to both the genera (Chowdhury, 1936).

Holden reported vertical gum ducts in *Dipterocarpoxylon burmense*. She saw what seems to have been disorganized cells and took them to be vertical gum ducts; certainly no vertical gum duct occurs in the sections I have studied (Pl. XX, No. 4). Gupta (1935) also reported the absence of vertical gum ducts. Unfortunately Kräusel, accepting Holden's accounts, classified this wood more near *Shorea* and *Hopea* than *Dipterocarpus*, all of which belong to the Dipterocarpaceae. Bancroft (1934), however, rejected it as Dipterocarpaceous and suggested affinity with the Meliaceae. Gupta (1935) suggested affinity with Ebenaceae and Anacardiaceae, but had he compared the parenchyma in detail he would no doubt have limited it to the Anacardiaceae and indeed to only a few genera near *Gluta*.

In Colani's specimen from Indo-China again vertical gum ducts were reported. She gave a photomicrograph of one, which in my opinion proves

that it is not a duct but a hole produced by decay prior to fossilization (Pl. XX, No. 2). It lacks the epithelial cells (parenchyma cells) of a true gum duct. The horizontal ducts in the rays reported by her are, however, real.

The reasons why *Glutoxylon assamicum* is identified with *Gluta* have been previously published (Chowdhury, 1936). The combination of characters, particularly apotracheal parenchyma bands at irregular intervals and horizontal gum ducts in the rays, occur in no other group. Vertical gum ducts were reported in two of the specimens mentioned in this paper, namely *Dipterocarpoxylon burmense* and *Dipterocarpoxylon annamense*, but I believe that both are mistakes of interpretation.

TABLE I

Showing various specimens referred to Glutoxylon burmense *(Hold.) n. comb.*

Serial number.	Specimen and original name.	Locality.	Age.
1.	*Dipterocarpoxylon burmense*, Holden 1916 *Irrawadioxylon burmense*, Gupta 1935 *Glutoxylon burmense*, Chowdhury 1950	Gwedindon, Sagaing District, Burma	Pliocene
2.	*Dipterocarpoxylon annamense*, Colani 1919 *Glutoxylon annamense*, Chowdhury 1950	Basin du Dadung, Indo-China	Tertiary
3.	*Dipterocarpoxylon* species, Sen 1930 *Glutoxylon* species, Chowdhury 1950	Lalmai Hills, Bengal	Pliocene
4.	*Glutoxylon assamicum*, Chowdhury 1936 *Glutoxylon burmense*, Chowdhury 1950	Nailalung, Assam	Miocene
5.	*Glutoxylon burmense*, Chowdhury 1950	Cachar Hills, Assam	Miocene
6.	*Glutoxylon burmense*, Chowdhury 1950	Laterite of Ranigunj coalfield, Bengal	Miocene to Pleistocene

V. DISTRIBUTION

Most of the recent species of *Gluta* are confined to South-east Asia, including Burma, Siam, Malaya, Indo-China, and as far north-east as Hainan Island, but two species occur isolated, one *G. turtur* in Madagascar and the other *G. travancorica* on the extreme south-west coast of India. It occurs mostly in evergreen forest from the sea-level to low hill-tops. Its northern limit is Hainan Island, its south in Java, and east in Celebes. *Melanorrhoea* is confined to the area where the bulk of the *Gluta* species occur, being absent in Madagascar and India.

The fossil species (Table I) occur not only in Burma and Indo-China, their present home, but also in India—Bengal and Assam—about 1,000 miles from the present main area. Clearly the genus has disappeared from the latter regions, but whether as a result of migration or as a result of extinction over a part of its range we cannot yet say. This is not in accordance with the

views of certain paleobotanists. For example, Krystofovich (1929) says: 'In the south, the flora has developed quite unmolested ever since its first descent from its cretaceous ancestors, except in response to slight changes in temperature and humidity which have taken place during this period. This is proved by fossil documents found in Borneo, Sumatra, Java, the Philippines, and other tropical countries. . . .'

VI. Acknowledgements

My grateful acknowledgements are due to Mrs. Sahni, Birbal Sahni Institute of Paleobotany, Lucknow, for allowing me to examine Holden's original specimen. Thanks are due to Mr. K. N. Tandan, Forest Research Institute, Dehra Dun, for taking some photomicrographs. I am grateful to Sir Edward Salisbury, F.R.S., Director, Royal Botanic Gardens, Kew, for allowing me the facilities to work in the Garden, and to Mr. W. N. Edwards, Keeper, Geological Section, British Museum, South Kensington, for the facilities of his library. I am very grateful to Professor T. M. Harris, F.R.S., University of Reading, for his help in the writing up of this paper.

Literature Cited

Bancroft, H., 1933: A Contribution to the Geological History of the Dipterocarpaceae. Geol. För. Stockh. Förh. lv. i. 59–100.

Chowdhury, K. A., 1936: A Fossil Dicotyledonous wood from Assam. Ann. Bot. Lond., l. cxcix. 501–10.

—— 1942: Paleobotany in India, III. J. Indian Bot. Soc., xxi. iii–iv. 222.

Colani, M., 1919: Sur un *Dipterocarpoxylon annamense* Nov. Sp. Tertiaire supposé de L' Annam. Bull. Surv. Geol. de L' Indo-Chine, iv. 1–8.

Edwards, W. N., 1931: Fossilium catalogus, II. Plantae, Pars 17. Dicotyledones (Ligna).

Gee, E. R., 1932: The Geology and Coal Resources of the Ranigunj Coal Field. Mem. Geol. Surv. India, lxi. 1–342.

Gupta, K. M., 1935: Critical remarks on *Dipterocarpoxylon burmense* Holden: *Irrawadioxylon*. Gen. Nov. Proc. Indian Acad. Sci. i. ix. 549–55.

Holden, R., 1916: A Fossil Wood from Burma. Rec. Geol. Surv. India, xlvii. iv. 267–72.

Kräusel, R., 1922 *a*: Fossile Hölzer aus dem Tertiär von Süd-Sumatra. Verh. Geol-Mijnb. Genoot. Ned. Kolon., v. 266.

—— 1922 *b*: Über einen fossilen Baumstamm Von Bolang (Java). Proc. Sec. Sci., xxv. 14.

Krystofovich, A. N., 1929: Evolution of the Tertiary Flora in Asia. New Phytol. xxviii. iv. 310–11.

Sen, J., 1930: Fossil Wood of the *Dipterocarpoxylon* type from the Lalmai Range in Comilla, Bengal. Quart. J. Geol. Soc. India, ii. 139–41.

EXPLANATION OF PLATE XX

Illustrating K. A. Chowdhury's article on 'Some More Fossil Woods of *Glutoxylon* from South-east Asia'

Glutoxylon burmense (Hold.) n. comb.

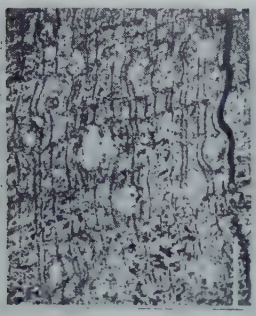
Fig. 1. Photomicrograph of cross-section given by Colani. Note a fusiform ray on the right-hand side marked *f.r.* (×10.)

Fig. 2. Another cross-section by Colani, showing the so-called vertical gum duct, *v.d.* (×75.)

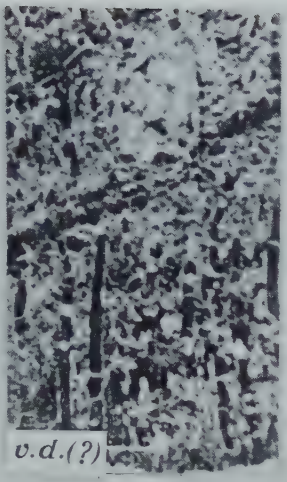
Fig. 3. Colani's photomicrograph of a cross-section, showing many spots like the so-called vertical gum ducts given in Fig. 2. These are actually holes produced by decay before fossilization. (×37·5)

Fig. 4. Cross-section of Holden's original specimen. Note distribution of vessels and apotracheal parenchyma cells. (×15.)

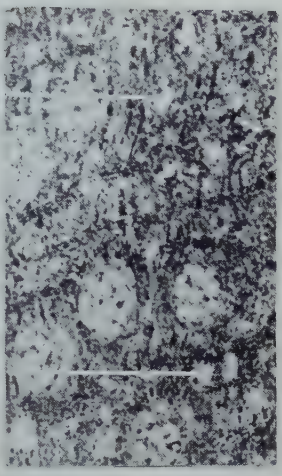
Fig. 5. Tangential section of Holden's original specimen. Note the fusiform ray (*f.r.*) with horizontal gum ducts and the shape and size of the uniseriate ray. (×200.)



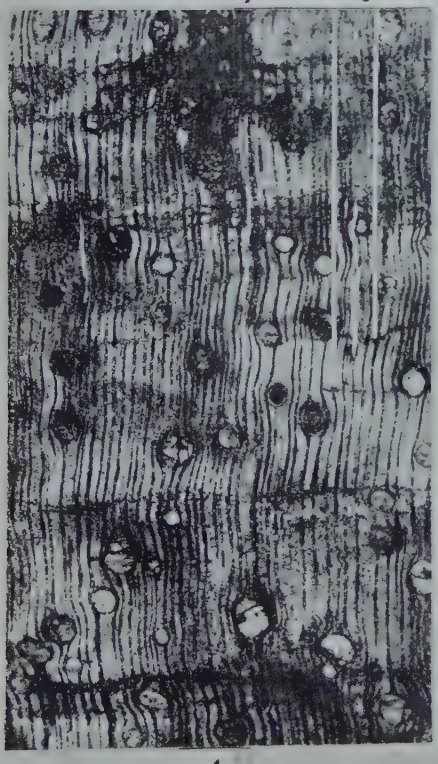
1



2



3



4

5

K. A. CHOWDHURY—*GLUTOXYLON*

The Suction Potential of Plant Cells and Some Related Topics

BY

D. C. SPANNER

(*Department of Botany, Imperial College of Science and Technology*)

With eleven Figures in the Text

Abstract

This paper is a theoretical discussion of the concept of suction potential or suction pressure as applied to plant cells, especially with reference to the possible occurrence of 'active' water-secreting mechanisms. Following an attempt to define what is meant by suction potential there is a discussion of the distinction between 'active' and 'passive' agencies in relation to water movement and a tabulation of such agencies.

Some of the less commonly considered mechanisms are discussed in some detail, and an attempt is made to evaluate how effective they might be in producing increments of turgor pressure. The conclusion is reached that if active mechanisms are operative in cell-water relations, then suction potential cannot adequately be defined in the ordinary way—in fact the 'water-absorbing effort' of a cell cannot be completely specified in terms of pressure alone.

There are appendixes on the possible role of frictional or contact electrification in cell physiology; on the increase of permeability of the plasma membrane to ions and water caused by electrical forces; and on the energy requirement of active water-secreting mechanisms.

Introduction

WATER is such an important constituent of all actively living organisms that it is not surprising that the study of its economy has attracted considerable attention. The typical vacuolated plant cell provides an attractive and apparently simple object for this study, the cellulose wall adding further to its suitability. As a result a classical theory has grown up in plant physiology based on this typical model and capable of explaining so much of the behaviour of plant tissues that only fairly recently has its adequacy been seriously challenged. For a long time the movement of water between cells was attributed solely to the osmotic properties of their vacuolar sap. Then in 1916 a considerable advance was made by Ursprung in the recognition that other factors besides osmotic pressure were involved—in particular, the elastic pressure of the cell walls. This gave rise to the conception of suction force or suction pressure, defined as the difference between the osmotic pressure of the cell sap and the elastic back-pressure of the cell walls. This resulted in a much more satisfactory interpretation of the water economy of plant

[Annals of Botany, N.S. Vol. XVI, No. 63, July, 1952.]

organs, and for many years this simple theory—which may well be called classical—held sway. Since about 1936, however, when Bennet-Clark published his first paper on the subject, the theory has been subjected to a good deal of criticism, the chief point at issue being whether or not active 'pumping' mechanisms are involved in water movement in addition to passive forces on which the classical theory is based. Bennet-Clark and others have produced a good deal of evidence for the existence of such 'active' mechanism (see, for instance, the bibliography in J. Levitt, 1947, or Crafts, Currier, and Stocking, 1949), and further support has come from an array of different observations —the secretion of water from hydathodes, the connexion between root pressure and respiration, the dependence of salt movements on oxygen supply, and the well-known pumping activity of many animal membranes, to mention only a few. Indeed, in view of so many well-attested phenomena it would seem surprising if active agencies were *not* in general involved in the ordinary water movements of plant tissues.

Definitions

Before proceeding further it may be as well to define carefully the meaning of our terms, for however difficult it may be to be precise in *measuring* physiological quantities, there is little excuse for lack of precision in *defining* them. Calling the property which governs the direction of water movement in a system *at uniform temperature* the 'suction potential' (after H. R. Oppenheimer, 1930) or the 'suction pressure', we can define it in two ways. The basic idea being that of pressure, we can define the suction potential of a cell (or system of cells) as the excess hydrostatic pressure to which it must be subjected to bring it into equilibrium with pure water.[1] This definition has the advantage of directness—in fact it has occasionally even formed the basis for the experimental determination of suction pressure[2] (T. A. Bennet-Clark and D. Bexon, 1940).

A second way of defining suction potential is by means of the concepts of (equilibrium) thermodynamics. Let μ_w^0 be the chemical potential[3] of pure water at the temperature and pressure of the cell (i.e. immediately *outside* the cell), and let μ_w be the chemical potential of water inside the cell. Let V_w^0 be the molar volume of water (i.e. the volume of one gramme-molecule). Then the suction potential π may be defined by the equation

$$\pi = \frac{(\mu_w^0 - \mu_w)}{V_w^0}. \qquad (1)$$

[1] Strictly the pressure of this water should be specified, but this is not a point of practical significance.

[2] The curves given by these authors, extrapolated to zero volume of expressed juice, really give the value of the lowest suction pressure of the leaf cells, in accordance with the above definition. However, as the cell walls approach infinite rigidity—as in effect these authors state —this value becomes equal to the hydrostatic pressure at full turgor.

[3] That is, $V_w = (\partial V/\partial n_w)_{T,P,n_i}$, where V is the volume of the cell and n_w is its water content in moles, the subscript n_i denoting that all other constituents are kept unchanged in amount while water is added. The chemical potential of the water (μ_w) is likewise the partial molar Gibbs free-energy, i.e. $\mu_w = (\partial G/\partial n_w)_{T,P,n_i}$, where G is the Gibbs free-energy of the cell.

Should appreciable changes in total volume occur when the cell takes in water, this equation must be replaced by the exact form

$$\int_{P_0}^{P_0+\pi} V_w \, dP = \mu_w^0 - \mu_w, \tag{2}$$

where P_0 is the pressure of the surroundings and V_w is now the *partial* molar volume[1] of water in the cell. However, since V_w is nearly constant and equal to V_w^0 in all ordinary cases, equation (1) is quite adequate. A further practical simplification may be made by replacing the chemical potentials μ with the much more convenient vapour pressures p. When this is done we get very closely

$$\pi = \frac{RT}{V_w^0} \log_e \left(\frac{p_w^0}{p_w} \right), \tag{3}$$

R being the gas constant, T the absolute temperature, and p_w^0, p_w the aqueous vapour pressures in equilibrium with pure water and with the cell respectively. Equation (3) is, of course, exactly similar to the well-known one for osmotic pressure.

Passive and Active Agencies

At first sight the above alternative definitions of suction pressure seem quite satisfactory, and indeed so long as the simple classical theory of water relations holds they are. But as soon as the possibility of active pumping mechanisms arises they become inadequate. Before discussing why this is so, however, it would clarify matters to state precisely just what is intended by the use of the words 'active' and 'passive', and the distinction between them.

From one point of view then a passive agency may be defined as one which is able to produce a true equilibrium, while an active one can lead only to a stationary state. When water is drawn into a cell by osmosis, causing a rise in pressure, the process goes on until the growing pressure balances the osmotic forces and equilibrium results. The chemical potential (or alternatively the activity) of the water is then the same both inside and outside the cell. To maintain this condition no expenditure of energy is necessary. Exactly the same reasoning would hold if the hydrostatic pressure were raised —water would escape until a new equilibrium resulted. Osmosis and hydrostatic pressure are therefore passive, or we might say thermodynamic, agencies.

On the other hand, any mechanism which is able to move water *against* a gradient of chemical potential is an active one. To do this requires the expenditure of energy. When such a mechanism moves water into a cell, the pressure rises until eventually it brings the influx to an end. The state reached, however, is not an equilibrium, since so long as the permeability to water remains it can only be maintained by the continuous expenditure of free energy, or, broadly speaking, by the performance of work. It is in fact a *stationary state.*

[1] See note on previous page.

Of course, the actual movement of water always involves the doing of work, but whereas with a passive mechanism the work is done at the expense of the free energy of the system, with an active one external energy, ultimately, must be drawn on.

From one point of view therefore the difference between active and passive agencies can be stated thus: an active agency can only maintain a turgor pressure so long as it possesses a continuous supply of energy, whereas with a passive one no energy expenditure is involved. The former leads to a stationary state, the latter to an equilibrium.

Looking now at the cell from a molecular point of view a different idea can be gained of the distinction. In a true equilibrium molecules will certainly be moving both into and out of the cell, but the movement will be entirely random. Over any element of area of the cell surface however small the net outward or inward movement of molecules or energy during a sufficient interval of time will be zero. This means of course that the forces brought into play are uniformly distributed, just as with a hydrostatic pressure. In the case of a stationary state, however, we have a different picture. Here the movement is *ordered* in some way or other and not entirely random. We may, for instance, find that the forces influencing water movement are not uniformly distributed over the permeable surface of the cell, so that when opposed by a hydrostatic pressure they are able to force water in at some points while it leaks out at others. This is perhaps always the case in electro-osmosis, or with mechanisms based on surface tension. The ordered movement here is then the circulation of water into and out of the cell by different paths, and this of course implies work.

Another method by which a stationary state can be maintained is illustrated by Osterhout's and Murray's anaphoresis. In this, a substance X diffusing from its aqueous solution through a suitable membrane into a weaker solution carries water with it. If the weaker solution were subjected to pressure the influx of water could presumably be prevented and its molecular movement across the membrane be made entirely random. The ordered movement here, however, is the continuous one-way diffusion of the substance X into the weaker solution. If the solute on its arrival in the latter were converted to a substance Y which did not possess its ability to act as a water carrier, and if this diffused back across the membrane where it was reconverted to X, then we should have a mechanism capable of maintaining a stationary state so long as it possessed the necessary energy supply to promote the chemical reactions. The 'circulation' would be, not of water, but of X and Y. These points will be discussed again below in connexion with anaphoresis and electro-osmosis.

One further distinction between active and passive mechanisms remains to be mentioned. When a cell is in true equilibrium under the influence of passive forces, its state (e.g. its water content) is quite independent of any properties other than thermodynamic ones, a fact already implied in equation (1). This, of course, is not true of the *rate* of attainment of equilibrium, but

only of the equilibrium state itself. The 'suction potential' of such a cell can therefore be completely defined thermodynamically.

With an active mechanism, however, with its requirement or ordered movement of some kind, the steady state will depend also on what may be called kinematic properties—for instance the viscosity or electrical or thermal conductivity of the cell contents, membrane substance, or surroundings. This means in fact that the 'suction potential' of the cell cannot be defined without reference to these properties, and it is this fact which renders the definitions attempted above logically inadequate. Further reference will be made to this later.

Before enumerating the various agencies concerned with water movement it may be helpful to summarize the differences between active and passive mechanisms as follows:

1. Passive mechanisms are capable of producing a true equilibrium; active ones only a stationary state, maintained by the continuous expenditure of energy.
2. Passive mechanisms can move water only down a gradient of chemical potential or activity; active ones can do so in either direction.
3. With passive movement work is done at the expense of the free energy of the cell; with active movement external energy, ultimately, must be drawn upon.
4. The forces on the water molecules are always distributed uniformly across the permeable surface in the case of passive mechanisms; they may be non-uniform in the case of active ones.
5. The final state reached will not depend on kinematic properties (such as viscosity, conductance, &c.) where an equilibrium is concerned; with a steady state it will.

Agencies causing Water Movement

The agencies concerned in the transport of water in plant cells may now be classified. Dealing first with passive agents, these are:

1. *Hydrostatic pressure*, arising from the elasticity of the cell walls, the influence of neighbouring cells, surface tension, or in some cases gravity.
2. *Imbibition.*
3. *Osmosis.*
4. *Electrostatic pressure.* This is added here merely as an interesting theoretical possiblity. Suppose there is a continuous lipoidal membrane, and that by some means or other—perhaps diffusion or the adsorption of a monolayer—a potential difference has been established across it. Then if it is assumed that water molecules in contact with the membrane are liable to frictional or contact electrification by the transfer of an electron (a fairly reasonable suggestion) this constitutes a passive, non-osmotic mechanism capable of maintaining a turgor pressure without the expenditure of energy. Some water molecules in the circumstances would acquire an electric charge

which, though perhaps very minute when averaged over the whole number of charged and uncharged molecules, would nevertheless affect the chemical—or rather the electrochemical—potential appropriate to equation (1). On the whole it does not seem likely that frictional or contact electrification would play much part, the total amount of electricity involved being probably far too small.

The active agencies may now be considered.

5. *Surface tension.* Imagine a pore *AB* through the cell membrane (Fig. 1). If a solute capable of lowering the interfacial free energy between the water and the membrane substance (which doubtless possesses a measure of fluidity) be added to the side *A* it will be adsorbed on to the surface of the pore on this side. As a result the layer of fluid adjacent to the pore wall will behave like a cylindrical rubber balloon whose walls are suddenly weakened at one end. A movement of the surface layer in the direction *A* to *B* will therefore take place dragging the fluid vein in the same direction. Of course, the active substance will be carried across the membrane as well—providing a mechanism which may sometimes explain the rapid penetration of such substances into cells[1]—and eventually the process will come to an end. However, if through some metabolic activity of the cell the active substance is destroyed as it enters or leaves the process would continue (Fig. 2).

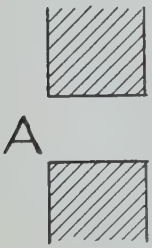


FIG. 1.

The mechanism does, of course, necessitate definite pores in the membrane, and these must be of adequate size and length in comparison with the molecules of the active substance. It therefore has a questionable application to the case of the plasma membrane. With regard to the nature of a possible 'carrier' it is interesting to recollect that conditions at an oil-water interface are rather different from those at an air-water interface. Thus inorganic salts usually raise the surface tension of water against air, but W. C. McC. Lewis[2] (1909) found that KCl and $BaCl_2$ appreciably lower the interfacial tension against petroleum oil. Sucrose, too, causes a lowering and the salts of fatty acids are more effective than the acids themselves—a reversal of the order found against air. Generally speaking (Freundlich, 1926) substances such as chloroform, ethyl alcohol, and benzene which have a high solubility in lipoids, although they may have a considerable effect on the air-water interface, have little or none on an oil-water one, for which it would seem in general that the substance should have a greater solubility in the phase of higher surface tension.

[1] Provided, of course, that they are active at an oil-water as opposed to a water-air interface. The mechanism recalls the experiments of Van den Honert (1932) on the rapid spread of potassium oleate along an ether-water interface. This rapid spread would cause streaming in the boundary layers of the two liquids.

[2] 0·0125 M KCl lowered it from 52 to 50·4 dynes/cm. and 0·062 M $BaCl_2$ from 45·81 to 40·99; M/6 sucrose lowered it 17 per cent.

Salts probably act by altering the zeta-potential of the interface, but the mechanism by which non-electrolytes like sucrose act must be different. Surface-tension effects may have had some bearing on the results observed by Bennet-Clark and Bexon (1946) with onion protoplasts transferred between isotonic sucrose and electrolytes. It might be surmised that hydrogen and hydroxyl ions would be particularly effective, and in their case the conversion to a non-active form (according to the scheme of Fig. 2) would require only the presence of buffering substances on one side of the membrane. Such a mechanism seems quite feasible.

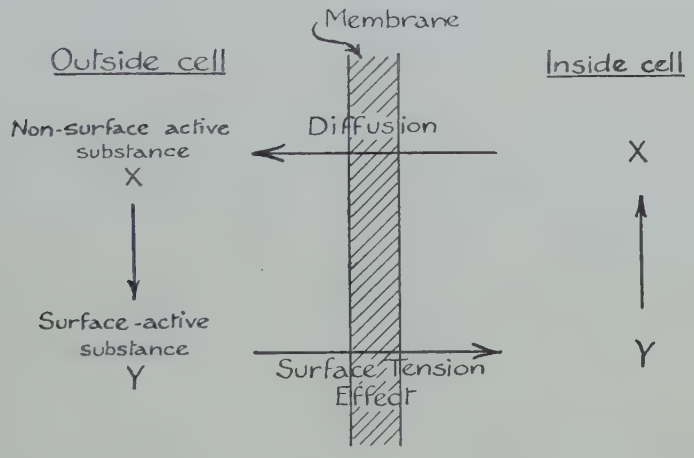


FIG. 2.

A very simple theoretical treatment can be given as follows. Consider a pore of length l and radius r, and let the difference in interfacial tension at its ends be $\Delta\gamma$. Suppose this produces movement of the column through the pore at a velocity v, this being considered constant over the cross-section except at the periphery, where it falls to zero over a boundary layer of thickness t. Then the volume flow will be given to a first approximation by

$$Q = v\pi r^2, \tag{4}$$

and equating the force on the column to the viscous resistance we shall have

$$F = 2\pi r\,\Delta\gamma = \eta\frac{v}{t}2\pi rl, \tag{5}$$

η being the viscosity.

Eliminating v we get

$$Q = \frac{\pi r^2 t}{\eta l}\Delta\gamma. \tag{6}$$

Assuming that the flow of water is counterbalanced by a hydrostatic pressure

P, we shall arrive at a steady state with an equal and opposite flow superimposed on the above and given approximately by Poiseuille's formula:

$$Q = \frac{\pi r^4 P}{8\eta l}. \tag{7}$$

Solving for P we find

$$P = \frac{8t}{r^2}\Delta\gamma. \tag{8}$$

This equation is not a complete description of the effect in question. To give this we should have to include factors describing how the surface-tension difference $\Delta\gamma$ was maintained, and if this were by a mechanism such as is depicted in Fig. 2, it would naturally involve kinematic properties such as the diffusion coefficient. A rather similar consideration applies to equation (13).

Let us, however, try to obtain an estimate of the order of magnitude involved. Taking the diameter of a water molecule as $2{\cdot}5\times10^{-8}$ cm., assume that t is twice this diameter and r ten times. This will give a fairly large pore, molecularly speaking. Then for a difference in interfacial tensions of only 1 dyne/cm. we have

$$P = \frac{8\times5{\cdot}0\times10^{-8}}{(25\times10^{-8})^2}\times1{\cdot}0 \text{ dynes/cm.}^2$$

$$= 6{\cdot}3 \text{ atmospheres.}$$

This is surprisingly large. The calculation is, of course, open to very many objections, but it does serve to show that surface-tension effects might reach quite high magnitudes. In practice t might well be greater than the value taken and r less. Of course, as the pore size is reduced it becomes questionable whether the usual values of γ apply; also the values of r appropriate to equations (4), (5) and (6) and (7) will begin to diverge; nevertheless the result is striking.

6. *Anaphoresis.* This is the name given by Osterhout and Murray (1939) to the transport of water by trichloracetic acid in the system aqueous acid: guaiacol: water. The acid increases the solubility of water in the guaiacol, and as a consequence water is transferred from left to right by the diffusion of the acid. How such a system could give rise to a steady turgor pressure has been discussed earlier on. The process recalls that observed by Flusin (cf. Freundlich 1926). He found that when a substance such as caoutchouc, which swells at different rates when immersed in various organic liquids, is placed as a membrane between two such liquids, that there is a transfer of liquid from the side containing the more rapidly swelling liquid to the other. One has only to imagine a component common to both liquids to see that this would probably be carried across the membrane in the same direction, and the parallelism with anaphoresis is obvious. Of course, such a transfer as Flusin observed would obtain with a porous membrane separating fluids of

different molecular weights merely on account of their different diffusion rates; but Flusin's data show that the rate of liquid transfer across a caoutchouc membrane is proportional rather to the swelling velocity, and that this depends on some additional factor besides the molecular weight of the liquid and the total amount of it which can be absorbed by the membrane substance.

Flusin observed a similar parallelism between passage of water across the membrane and swelling velocity in the case of acid solutions separated from water by pig's bladder, but in this and other cases involving electrolytes, electro-osmosis almost certainly plays a large part.

Anaphoresis and related mechanisms would seem to require the diffusion of relatively large amounts of a second substance in order to transport water, and for this reason the likelihood of their playing a large part in cell physiology is diminished.

7. *Electrolysis.* Suppose a mechanism exists for maintaining a potential difference across a permeable membrane. Then salts in the pores of the membrane will tend to suffer electrolysis, and owing to the different degrees of hydration of their ions a transport of water will take place. Suppose as a first approximation that Stokes's law can be applied to the hydrated ions. Then if u_+ and u_- are the ionic mobilities, and if r_+ and r_- are their effective radii, we shall have, assuming that both ions are univalent and that the concentration is not too great,

$$F = 6\pi\eta r_+ u_+ = 6\pi\eta r_- u_-, \tag{9}$$

where F is the frictional force on an ion moving in unit field. It follows from (9) that, as a first approximation at any rate, the product ru is the same for the two ions.

Now let the potential gradient be dE/dx and the number of salt molecules N per unit volume. Then the ionic velocities will be $u_+(dE/dx)$ and $u_-(dE/dx)$ and the volume flow (most of which will be of water) towards the cathode per unit area per second will be given by

$$\begin{aligned} Q &= \left(u_+ \frac{dE}{dx}\right) N \tfrac{4}{3}\pi r_+^3 - \left(u_- \frac{dE}{dx}\right) N \tfrac{4}{3}\pi r_-^3 \\ &= \tfrac{4}{3}\pi N \frac{dE}{dx}(u_+ r_+^3 - u_- r_-^3). \end{aligned} \tag{10}$$

Again F, the frictional force on a univalent ion moving in unit field, is numerically equal to ϵ, the electronic charge. Hence we have

$$\epsilon = 6\pi\eta r_+ u_+ = 6\pi\eta r_- u_-.$$

Substitution in (10) gives

$$Q = \frac{2}{9}\frac{N\epsilon}{\eta}\frac{dE}{dx}(r_+^2 - r_-^2). \tag{11}$$

Suppose now that a back pressure (P) be applied to produce a stationary condition. Then by Poiseuille's law we shall have:

$$\text{Flow per unit area of pore} = Q = \frac{1}{\pi a^2}\frac{\pi a^4}{8\eta}\frac{dP}{dx}, \tag{12}$$

where a is the radius of the pores. Eliminating Q we get

$$\frac{dP}{dx} = \frac{16}{9}\frac{N\epsilon}{a^2}\frac{dE}{dx}(r_+^2 - r_-^2),$$

or integrating,

$$\Delta P = \frac{16}{9}\frac{N\epsilon}{a^2}\Delta E\,(r_+^2 - r_-^2), \tag{13}$$

where N is assumed constant, and ΔP and ΔE are the differences of hydrostatic pressure and electrical potential between the two sides of the membrane.

This simple theory shows that the effect ΔP will be proportional to the membrane potential ΔE, to the difference in size of the hydrated ions, and inversely to the square of the pore diameter. Since r is inversely proportional to u, ΔP will be greater as the ionic mobilities diverge more widely, and the higher pressure will develop on the side to which the slower ion migrates. In the case of, say, an organic acid the disparity in ionic radii will be so great that r_+ may be neglected. Taking a as equal to five times r_- we can then get a very simple idea of the sort of magnitude to be expected. Let ΔE be 100 millivolts, and let the concentration of ions be one-tenth normal. Then $N\epsilon$ will be 9·65 coulombs per c.c. and we shall have

$$P = \frac{16}{9}\frac{9{\cdot}65}{25}\frac{100}{1000}\text{ joules per c.c.}$$
$$= 0{\cdot}7\text{ atmospheres.}$$

It seems unlikely from the above that electrolytic effects will be of much importance. The 'efficiency' is very low, since work done on ions of one sign actually opposes the pressure build up, and water transport is limited to the molecules closely investigating the ions. Further, the mobilities of anions in the membrane will commonly be very much reduced below their values in the free solution. A much more efficient mechanism is electro-osmosis, which is discussed below.

8. *Ion exchange.* A mechanism rather similar to the preceding depends on ion exchange. Imagine a membrane permeable only to cations and separating solutions of an acid (say HCl) from a salt (NaCl). Initially, hydrogen ions will diffuse across the membrane at a rate faster than sodium ions, and as a result a potential difference will be set up which will eventually equalize the rates of passage of the two cations. This condition will, of course, involve the transport of water, since the two ions will be differently hydrated. An exact treatment of the problem is complex, but the following very rough method gives an estimate of the order of magnitude of the effect.

Assume that the potential difference across a membrane of thickness x is

E, and that the diffusion coefficients of the two similarly charged ions are D_1, D_2. Further, let the mobilities of the ions be u_1, u_2, and let their concentrations vary, in opposite directions, from c' on one side of the membrane to c'' on the other, c' and c'' being not too widely different.

Then the rate of diffusion of the slower ion across the membrane per unit area of pore will be roughly $D_1\left(\frac{c'-c''}{x}\right)$. But the potential gradient will cause an additional rate equal on the average to $u_1\frac{E}{x}\left(\frac{c'+c''}{2}\right)$. The total rate will therefore be

$$q_1 = D_1\left(\frac{c'-c''}{x}\right)+u_1\frac{E}{x}\left(\frac{c'+c''}{2}\right). \tag{14}$$

For the faster ion the potential gradient will tend to offset the diffusion, and we shall have

$$q_2 = D_2\left(\frac{c'-c''}{x}\right)-u_2\frac{E}{x}\left(\frac{c'+c''}{2}\right). \tag{15}$$

When the steady state has been reached, q_1 and q_2 will be equal. Calling $q_1 = q_2 = q$, and eliminating E between (14) and (15) we get

$$q = \left(\frac{D_1u_2+D_2u_1}{u_1+u_2}\right)\left(\frac{c'-c''}{x}\right). \tag{16}$$

Since $D = RTu/F$ for a univalent ion, F being the Faraday, it is clear that in this simple case the ions diffuse at a rate governed by the coefficient D_m given by

$$D_m = \frac{RT}{F}\left(\frac{2u_1u_2}{u_1+u_2}\right). \tag{17}$$

If now r_1 and r_2 are the effective radii of the hydrated ions, the volume flow per unit area of pore will be

$$Q = D_m\left(\frac{c'-c''}{x}\right)N(\tfrac{4}{3}\pi r_1^3-\tfrac{4}{3}\pi r_2^3), \tag{18}$$

where N is Avogadro's number and concentrations are in gramme ions per unit volume.

Using Poiseuille's formula as before to find the turgor pressure set up, we get

$$\Delta P = \frac{32\pi}{3}\frac{\eta N}{a^2}D_m(c'-c'')(r_1^3-r_2^3). \tag{19}$$

As a concrete example let the concentration difference be one-tenth molar, the ions concerned being H^+ and Na^+. Take η for water as 0·0101 poise, $N = 6{\cdot}02\times10^{23}$, $r_H = 0$, and $r_{Na} = 3\times10^{-8}$ cm.[1] Let the diameter of the

[1] Allowing about 14 molecules of water to the sodium ion. Since allowance must be made for water molecules *frictionally* drawn along this may be on the low side.

pores be about ten times that of a water molecule, i.e. 25×10^{-8} cm. Then since u_H is so much greater than u_{Na} we shall have very nearly

$$\begin{aligned} D_m &= \frac{2RT}{F}u_{Na} \\ &= \frac{2\times8{\cdot}31\times298}{96500}\times5{\cdot}19\times10^{-4} \\ &= 0{\cdot}266\times10^{-4}\ \text{cm.}^2/\text{sec.} \end{aligned}$$

Inserting these values in equation (19) we get

$$\begin{aligned} \Delta P &= \frac{32\pi}{3}\times\frac{0{\cdot}0101\times6{\cdot}02\times10^{23}}{(12{\cdot}5\times10^{-8})^2}\times0{\cdot}266\times10^{-4}\times0{\cdot}1\times10^{-3}\times(3\times10^{-8})^3 \\ &= 9{\cdot}4\times10^{5}\ \text{dynes/cm.}^2 \\ &= 0{\cdot}93\ \text{atmospheres.} \end{aligned}$$

Thus it might appear that the effect is small, bearing in mind that the concentration difference $(c'-c'')$ for the hydrogen ion has been taken far greater than it could possibly be in actual cell systems. On the other hand, the diffusion rates of positive ions are likely to be much higher (perhaps enormously so) through the pores of a negatively charged membrane (see Appendix II). The following considerations bring this out in a simple way. The mean thermal kinetic energy of a molecule in a direction perpendicular to the membrane is $\frac{1}{2}kT$, where k (Boltzmann's constant) $= 1{\cdot}38\times10^{-16}$ erg per degree. At normal temperatures (25° C.) this works out at about $2{\cdot}0\times10^{-14}$ erg. Now imagine that a short pore of the diameter taken above (i.e. 25×10^{-8}cm.) contains five univalent anions attached to its walls. Then the electronic charge ϵ being $4{\cdot}77\times10^{-10}$ electrostatic units, the negative potential in the centre of the pore can be calculated as

$$\frac{q}{Kr}=\frac{5\times4{\cdot}77\times10^{-10}}{81\times12{\cdot}5\times10^{-8}}=2{\cdot}4\times10^{-4}\ \text{e.s. units, or 70 mV.}$$

K (the dielectric constant) being assumed equal to 81, the value for water in bulk. The electrical work done on a univalent cation in bringing it to the centre of the pore is therefore

$$\begin{aligned} w &= 4{\cdot}77\times10^{-10}\times2{\cdot}4\times10^{-4} \\ &= 1{\cdot}1\times10^{-13}\ \text{erg,} \end{aligned}$$

which is about five times greater than the mean thermal component. Clearly it will act to aid diffusion, and in fact the speeding up will be much greater than at first sight appears owing to the necessity, broadly speaking, of deducting a fixed amount of energy (the activation energy of diffusion) from both amounts. This will naturally increase their ratio.

It may further be observed (see in a rather different connexion, T. Teorrel, 1938) that if the slower ion be present in large excess on both sides of the membrane, the diffusion rate appropriate to equation (19) becomes almost

that of the fast ion. Of course, the application of Poiseuille's law to such a case as the present one is highly questionable; a correction for 'slip' at the walls would undoubtedly have to be applied, and this would lower the effect. However, it may also be noted that the viscosity, which appears in equation (19), might easily be very much higher than the value for water. Ordinarily this would mean a proportionate reduction in D, so that ηD would not change, but in the special case of the hydrogen ion, where movement is usually considered to be partly, at least, by a form of Grötthus conduction, it is possible that D might remain high even with a considerable rise in viscosity.

It may be remarked that the present mechanism does not necessarily require the existence of pores as ordinarily understood. The membrane

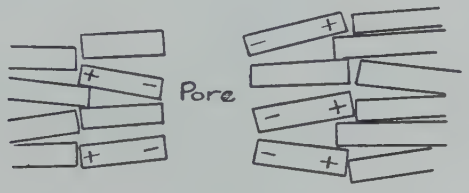


FIG. 3.

might be lipoidal and quite continuous in nature. The effectiveness of the mechanism judged, for instance, by the 'turgor pressure' it was able to maintain, would then depend on the relative speed with which water-carrying ions were able to pass in across the membrane and water molecules to escape out. If it be objected that the permeability to water of the plasma membrane is much greater than its permeability to salts, the answer might be given that the latter may be conditioned by the inability to penetrate of, say, the anions, and that where the cations are free to pass on their own (as is assumed in the present case) their rate of doing so might be very high, accelerated as it is (much more than in the case of water) by the membrane charge and the membrane potential (see Appendix II). We may conclude therefore that ion exchange may possibly be an important cause of water movement in cells.

9. *Electro-osmosis.* This is probably the best known of the active agencies concerned in water movement. It differs from the preceding ones in that a new factor is necessarily involved, the electrically charged state of the pore walls. This state may arise in several ways. In the first place the membrane substance may be ionogenic itself, and split off, say, hydrogen or hydroxyl ions. Secondly, the membrane may preferentially adsorb ions of one sign already present in the fluid phase. Thirdly, with special systems frictional or contact electrification may occur, involving the transfer of bare electrons. Finally, if the molecules comprising the membrane substances are polar, they may be oriented in such a way as to produce an electric field within the pores (Fig. 3), or the same result could follow from the oriented adsorption of dipoles (such as water or amino acids) from the fluid.

To digress for a moment, consider a thin membrane composed of non-polar molecules, and imagine a pore of radius $(r - \frac{1}{2}l)$ to be lined with oriented polar molecules of dipole moment μ (Fig. 4). Let the polar nature result from the separation within the molecule of charges $\pm q$ by the distance l, and let the number of polar molecules per unit of circumference of the pore be n.

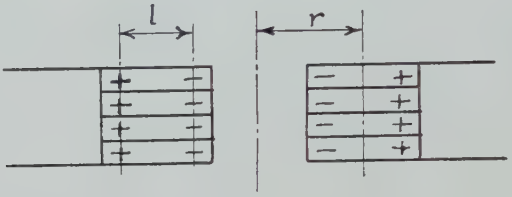


FIG. 4.

Then the electrical potential at the centre of the pore will be given by

$$V = \frac{n2\pi rq}{K(r+\frac{1}{2}l)} - \frac{n2\pi rq}{K(r-\frac{1}{2}l)}, \tag{20}$$

since the total positive and negative charges are $n2\pi rq$ and they are concentrated at distances $(r+\frac{1}{2}l)$ and $(r-\frac{1}{2}l)$ from the centre respectively. K is the dielectric constant of the fluid in the pore.

Equation (20) can be reduced nearly to

$$V = -\frac{2\pi n\mu}{Kr}, \tag{21}$$

μ, the dipole moment, being equal to ql.

This rough equation shows that small pores can be considerably affected by adsorbed water molecules. For if r is taken as equal to 10^{-7} cm.[1] and μ as $1{\cdot}84\times10^{-18}$ for water, the potential V works out at about

$$2\pi\times\frac{10}{2{\cdot}5\times10^{-8}}\times\frac{1{\cdot}84\times10^{-18}}{81\times10^{-7}}\text{ e.s. units} = 170\text{ mV}$$

for a pore about ten water molecules long.

This value has been calculated on the assumption that the dielectric constant for water in small pores is the same as in bulk, an assumption that may well be pessimistic.

Returning to the subject of electro-osmosis, it may be noted that whatever the origin of the effective charge on the pore walls (i.e. the zeta-potential), the net result is to bring about a difference in the numbers of mobile positive and negative ions[2] in the fluid vein, for even in the case of the adsorption of dipoles, the field so set up will tend to drive ions of one sign out of the pore

[1] About four times the diameter of a water molecule.

[2] Strictly, a difference in the total quantity of positive and negative electricity on these. For simplicity, differences in valency are here neglected.

and attract in ions of the other. Thus in every case the fluid vein becomes, in effect, charged.

If now a potential difference be applied to the ends of the pore, the ions of one sign will move one way and those of the opposite sign the other. In their movement they will exert a frictional drag on the water molecules. Were their numbers equal the net sum of all the elemental frictional drags would be zero, but since their numbers are unequal there will be an unbalanced force tending to move the vein as a whole, and in the direction in which the more numerous ions are moving. It is this unbalanced force setting the water molecules in motion which constitutes electro-osmosis.

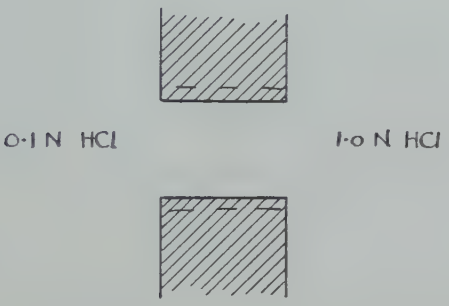


Fig. 5.

The effect of the movement of the vein will be to increase the speed of the more numerous ions relative to the pore walls, and to reduce, or even to reverse, that of the less numerous. Thus, if the resistance to motion of the fluid vein is small enough, both types of ion may even move in the same direction (relative to the pore) though at different speeds.

Consider now a very simple case of electro-osmosis (Fig. 5). Let the figure represent a pore in a membrane separating two concentrations of HCl, the substance of the membrane being negatively charged. Owing to the faster diffusion of the positive ion a difference of potential will be set up, the left-hand side becoming positive with respect to the right-hand side. As a result of the charge on the pore walls the fluid vein will contain more positive ions than negative, i.e. it will be positively charged. It will therefore experience an electrical force urging it bodily to the right.

It is instructive to consider rather more closely what happens in the above simple case. As soon as the solutions are put into contact with one another through the pore the hydrogen ions will begin to diffuse from right to left at a rate greater than that of the chloride ions, the difference being due partly to their inherently greater mobility and partly to the effect of the zeta-potential of the membrane, which increases their speed and concentration as they approach the pore. Very rapidly, however, a potential difference will be set up which will equalize the diffusion rates of the two ions, and a steady state will result. What is now happening to the water molecules within the

pore? If the membrane charge (as distinct from the potential across it) is adequate the vein will still contain more positive ions than negative. It will therefore be charged as a whole, and it will be situated in the electric field resulting from the diffusion potential. It would seem therefore at first sight that it must move, i.e. that so long as the concentration gradient of HCl exists there must be *electro-osmosis* of water to the right (superimposed, naturally, on the ordinary osmosis). This conclusion is of course wrong, as can be seen at once by considering the case in which the membrane is entirely impermeable to anions. The steady state here will be an equilibrium,[1] the cations being ultimately held back from further diffusion by the potential difference. However, the vein is still charged and is situated in an electric field; why does it not move? The reason is not far to seek, and lies in the fact that concurrent with the electrostatic force to the right there is an equal and opposite force to the left due to the osmotic effect of the ions causing the potential gradient. These two forces are quite inseparable and serve to cancel out *electro-osmotic* flow except under certain specified conditions. Obviously, therefore, the simultaneous occurrence of both a zeta-potential and a membrane-potential is not in itself sufficient to cause movement of the vein.

What, then, are the conditions necessary for electro-osmosis? They may be summarized as follows. Firstly, the fluid veins must contain unequal numbers of positive and negative ions, i.e. they must be charged. This is only another way of specifying that there must be a zeta-potential. It usually, but not always, implies a charge on the membrane (see above).

Secondly, there must be in general a potential difference between the two sides of the membrane.

Thirdly, this potential difference must not be equal to the steady value which would arise spontaneously were the vein left as the sole connexion between the two sides of the membrane. This last condition may be illustrated as follows:

Consider a pore (Fig. 6) containing both anions and cations neither equally nor uniformly distributed. Fixing attention on one particular section XX of the pore it is clear that positive and negative ions will be diffusing across it at rates depending on their diffusion coefficients (or mobilities) and concentration gradients. If now an electrical field be applied parallel to the axis of the pore, one kind of ion will be accelerated and the other retarded. It follows that there is one value of the field for which the two rates will become equal. If the field satisfies this condition at every section there will be no net transfer of electricity anywhere and the curve of electrostatic potential will be steady. This steady condition is clearly what would arise spontaneously were the vein acting alone as postulated above.

To justify the statement that in this state and in this only can electro-osmosis be said to be inoperative, it may be remarked that since the vein contains unequal numbers of opposite ions its movement *as a whole* (which can be taken as a definition of electro-osmosis) would seem to imply a net transfer

[1] i.e. provided the osmotic forces are suitably counter-balanced.

of electricity; so that it would be reasonable to say that where there is no net transfer there is no electro-osmosis. Further, although in the steady state the electrolyte is not exactly diffusing as an entity (the scarcer ion moving proportionately faster than the other), yet the frictional force exerted on the water by the ions is just the same as it would be if the two ions were of equal concentration and equal speed. For if f is the force required to move an ion through the water with unit velocity, n the number of ions per unit volume,

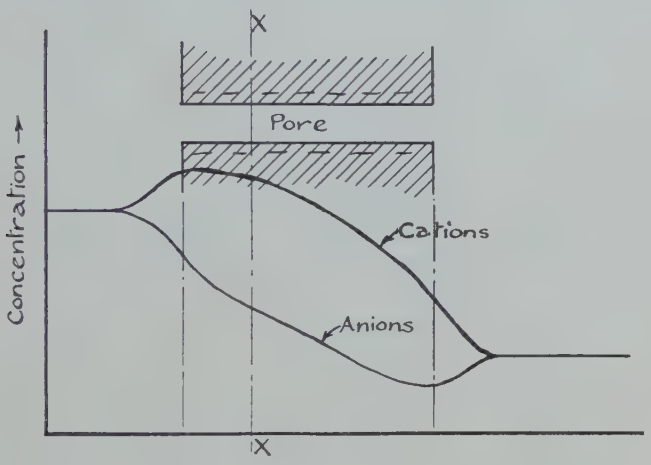


FIG. 6.

and v their velocity, then their frictional drag on the water per unit volume is given by

$$F = fnv$$

$$= fv,$$

where v is the number crossing unit area per second. Now in the steady state (and only in that) v is the same for both ions; hence so far as motive force on the water is concerned both ions might just as well be present at equal concentration and be moving at equal speed. But this would mean that the electrolyte was diffusing entirely as an entity, just like non-electrolytes. This again emphasizes that we are justified in asserting that electro-osmosis is not now taking place.

Viewed in this light it is clear that electro-osmotic movement will only take place if a potential difference other than its own 'free' value be applied to a pore, and that the direction of movement will depend on whether the actual potential difference (P.D.) is below or above this value.

As has often been remarked (see, for instance, Sollner, as quoted by Höber, 1945), a condition which leads to electro-osmosis arises when a membrane separating two solutions contains pores of different sizes or permeabilities. Fig. 7, after Sollner, shows a typical case.

The smaller pore, which we can assume to be impermeable to the chloride ion, sets up a greater potential difference than the larger one. Left to itself the larger pore would establish a P.D. under the influence of which hydrogen and chloride ions would pass through it at equal rates. However, the small pore allows relatively more hydrogen ions to pass and so raises the P.D. at the ends of the larger. As a result the ions in the latter experience an added electrical force, the special element of electro-osmosis coming in owing to the fact that there are more hydrogen ions to be driven back than chloride ions to be attracted forward, and this leads to a general movement of the vein to the left.

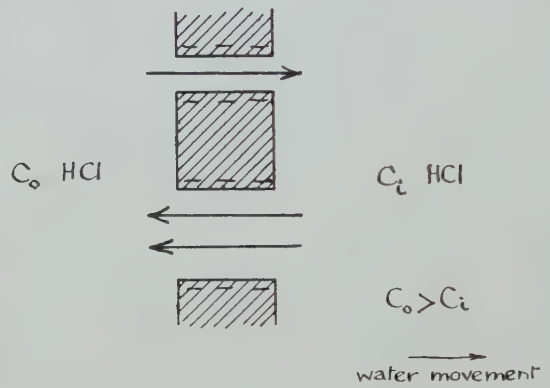


FIG. 7.

It should be remarked, however, that while the P.D. at the ends of the larger pore is maintained by the smaller at a value above the 'free' one, that at the ends of the smaller is concurrently reduced by the influence of the larger. Thus in the smaller pore there will be an electro-osmosis to the right.

The relative flows in the two directions would depend on several things: the proportion of large and small pores, their respective resistances to viscous flow, and the electrical conductivity of the solutions on either side. It would seem, in fact, that it is impossible to say in which direction the net flow of water would be without knowing more than is furnished by the details of Fig. 7. Such a set-up *might*, therefore, show either positive or negative movement, and this seems to cast a little doubt on the interpretations of direction advanced by Sollner (see Höber, 1945). It should be noted that in Sollner's experiment (quoted by Höber) the water movements through the two kinds of pore were isolated, so that he did not directly observe the *net* effect.

It is also worth remarking that it is not absolutely necessary, as is often stated, that there should be pores of different kinds present. Even a single pore[1] is active to a certain extent, since its electrical properties will vary from the centre to the periphery. Fig 8 shows what might be expected in such a

[1] This will, of course, necessitate a slight modification of the definition given above for the 'free' potential of a pore.

case. Here the peripheral region corresponds to the smaller pore of Fig. 7, and the axial to the larger. As in every case under discussion, of course, these movements must be considered as superimposed on the purely osmotic ones.

10. *Temperature gradients.* Two open dishes of water maintained side by side in a closed chamber will be at equilibrium with one another provided they are at the same temperature. If one be now raised in temperature by the

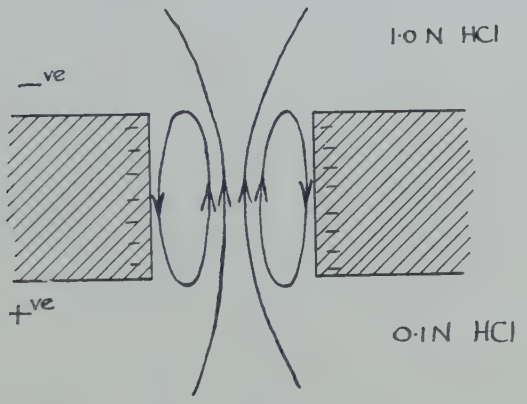


Fig. 8.

amount dT, water will begin to distil from it into the other. However, equilibrium, or rather a species of steady state, can be restored by raising the pressure of the water in the second dish—enclosing it, for instance, in a porous cylinder fitted with a piston. The condition for the 'pseudo-equilibrium' may be taken as equality of vapour pressure, and will be given by

$$\left(\frac{\partial p}{\partial T}\right)_P dT = \left(\frac{\partial p}{\partial P}\right)_T dP, \tag{22}$$

where the pressure on the water is P and its vapour pressure is p.

In the resulting state there will be a slow flow of heat through the vapour phase, but under certain conditions this will be low, i.e. the energy needed to maintain the system will be small.

The above situation may be compared to that existing in a cell or organism where metabolic energy maintains one portion at a slightly higher temperature than another. To see the order of magnitude of the pressure increase, we can proceeds as follows. It can be shown thermodynamically that $(\partial p/\partial P)_T = V_L/V_G$, where V_L and V_G are the molar volumes of water in the liquid and gaseous states respectively. Further, $(\partial p/\partial T)_P = L/(TV_G)$, where L is the molar latent heat of vaporization at constant pressure. Hence from (22) we get

$$\frac{dP}{dT} = \frac{(\partial p/\partial T)_P}{(\partial p/\partial P)_T} = \frac{L}{TV_G}\frac{V_G}{V_L} = \frac{L}{TV_L}. \tag{23}$$

Taking $L = 585$ cals. per gramme, $V_L = 1{\cdot}0$ c.c. per gramme, and $T = 20°$ C., we get

$$\frac{dP}{dT} = \frac{585}{293 \times 1{\cdot}0} \times 41{\cdot}31 = 82{\cdot}5 \text{ atmospheres per °C.}$$

This result is worth pondering; it indicates that *under suitable conditions* a temperature difference of only one-hundredth of a degree could induce water movement and so set up a pressure of nearly an atmosphere. The 'efficiency' of this process for converting heat into work is limited by the presence of at least one irreversible phenomenon, that of heat conduction. This would be very serious over such a short distance as the thickness of the plasma membrane, but temperature differences can obviously play quite an important part in such phenomena as root pressure and water of guttation.

It should, however, be added that equation (23) assumes that the criterion of the steady state is equality of vapour pressure. This may sometimes be so (see, for instance, O. F. Curtis, 1937), but it is not necessarily the case. It has to be remembered that where there are temperature differences the system is of necessity not in equilibrium, and the criterion of the steady state will vary with circumstances. For example, with a gas-filled 'cell' in a gaseous atmosphere at a different temperature the steady state (i.e. constancy of 'turgor pressure') will obtain when there is equality of pressure inside and outside if the 'plasma membrane' possesses large pores; if the pores are very small (even if the total membrane 'permeability' is the same), the condition becomes equality of frequency of molecular impacts—quite a different state of affairs. B. S. Meyer's (1945) remark that at constant external pressure 'increase of temperature has relatively little effect on the diffusion pressure of water' seems a little misleading. As a thermodynamic property the temperature dependence of diffusion pressure has surely quite a definite value—in fact differentiation of equation (1) gives it as

$$\left(\frac{\partial \pi}{\partial T}\right)_P = -\frac{1}{V_w^0}(S_w^0 - S_w), \qquad (24)$$

where S_w^0 is the molar entropy of pure water and S_w is the partial molar entropy of the water in the system, V_w^0 being assumed constant. What Meyer was referring to was the fact (see O. F. Curtis, 1937) that water seems to move very slowly *in the liquid phase* along temperature gradients. This can be reconciled with a possibly high temperature dependence of the diffusion pressure by the consideration noted above, that in a non-isothermal (and therefore non-equilibrium) system the diffusion pressure ceases to be the criterion of direction of water movement. All this serves to emphasize the difference between active and passive agencies, and of the need to know a good deal more about the system—in this case particularly the 'membrane'—before we can hope to estimate the energy necessary to maintain an 'actively' established turgor pressure (see Appendix 3).

Active Agencies and the Suction Potential

Having enumerated and discussed some of the mechanisms, both active and passive, which can cause water movements and establish turgor pressures in plant cells, it remains to consider the adequacy or otherwise of the definitions of suction pressure or potential given earlier on. So far as the passive mechanisms are concerned the two definitions are entirely satisfactory and nothing more need be added; but as soon as active ones enter into the water relations of the cell the definitions become inadequate. This can be seen at once from the fact that non-thermodynamic properties—such as viscosity

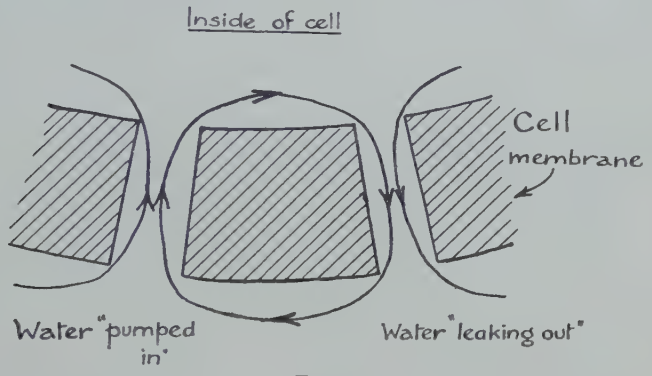


Fig. 9.

and electrical conductivity—appear in the formulae, and therefore any definition of suction potential in terms of equilibrium becomes impossible. But cannot the first mode of definition given above be altered to make it embrace stationary states as well as equilibria? Could it not, for instance, be made to read, 'The suction potential of a cell is the excess hydrostatic pressure to which it must be subjected so that in the presence of pure water it neither gains nor loses moisture'? The answer to this, unfortunately, is again 'No'; for consider how such a pressure could be applied. Imagine an 'active' cell immersed in fluid. As mentioned earlier, the maintenance of a turgor pressure by an active mechanism involves the continuous circulation into and out of the cell of some component, whether it be water itself or an electric current or something else. Now part of the path of circulation must be *outside* the cell (i.e. outside the plasma membrane). How can the cell be subjected to pressure (hydrostatic or osmotic) to perform the measurement without interfering with this portion of the path, and so altering the 'effort' of the mechanism? For instance, if water is being pumped in at some points and is leaking out at others, the energy expended will be used up partly in forcing water across the membrane and partly in conveying it parallel to the membrane between the points of entry and of exit (Fig. 9). If now the viscosity of the external plasmolyticum is increased (this need involve no change in its osmotic pressure) more of the energy will be expended

in the external path and less will be available for pumping water into the cell, which will consequently lose water. The same argument would hold if a solid surface be applied to the cell for the purpose of exerting a mechanical pressure on it. If an electro-osmotic process be at work, the extent to which the e.m.f. of one pore is transmitted to another and so enabled to cause electro-osmotic flow will clearly depend on the depth and conductivity of the fluid outside the membrane, and reduction of the latter—independent of any variation in tonicity—will be sufficient to cause the cell to lose water. The effect will only be important, of course, if the external path consumes an appreciable amount of the total energy. It may possibly be very much less resistive than the passages through the plasma membrane, though it has to be remembered that a cell wall closely invests the protoplast. But further, the pressure set up by the mechanism will depend on the relative resistances of the paths into and out of the cell. If the 'leakage' path be widened relatively to the 'entry' path (neither need lose their semipermeability), the cell will naturally lose water and take up a new stationary state.

Thus it would appear that whereas the power of a cell to attract water is dependent only on its classical 'suction pressure' so long as passive agencies alone are concerned, three factors at least are important in the case of active cells: the classical suction pressure, the properties of the external environment, and the detailed structure of the plasma membrane. To these a fourth might be added, the energy supply.

In practice, these considerations would lead to the conclusion that measurements of suction pressure by different methods would in general lead to different results, unless indeed active mechanisms play a quite insignificant role. The suction pressure in fact becomes not an unequivocally defined property; no magnitude can be logically assigned to it for an isolated cell, but only for a cell in an exactly specified environment. Further, it becomes thermodynamically possible for cells which are in 'equilibrium' with a given cell to be out of 'equilibrium' with one another. These observations may be taking an unduly pessimistic view, but they are worth making if only for theoretical interest.

Conclusion

This paper has attempted to deal with the question of the water relations of plant cells from a theoretical standpoint. A number—doubtless there are more—of mechanisms which might influence these relations has been discussed. Although the quantitative estimates of their importance are very sketchy indeed—chiefly because we have assumed large pores to which Poiseuille's law can be applied—they do serve to show that some of them may be of considerable importance. How the picture would change if we knew the laws governing flow of liquids through really minute pores, or through lipoid membranes, must remain a mystery till more experimental work has been done on the subject, but we know from experimental and theoretical investigations with gases that these laws are quite different for very small pores.

Hitherto in this paper little has been said about nomenclature. Should, however, it be demonstrated that cells do actively absorb water to any considerable extent it would seem advantageous to have a name for that part of their 'effort' which is thermodynamically defined, viz. the 'classical' or 'passive' component. This we have called the 'suction potential'—both because it is a *potential suction* and also because it is a potential in a well-established sense of the term. However, there are several other excellent terms in more current use, especially perhaps 'diffusion pressure deficit' and 'suction pressure'. For the net result of all the activities of the cell some more non-committal term is required—such as 'water absorbing effort'—since we have seen that the result can only be inadequately described in terms of pressure.

The problem of how to demonstrate the existence of active mechanisms is not an easy one, alternative explanations being available for so many observations which might be considered to establish it. For instance, the common criterion of energy-dependence (R. Höber, 1945) for active absorption of substances is not absolutely decisive, for a cell which began to lose water when its energy supply was cut off might do so because a change in protoplast structure allowed osmotically active solutes to leak out. Bennet-Clark's method of comparing the osmotic pressure of the vacuolar sap and the external solution seems one of the most promising, though hitherto it has always been open to the objection that the sap might not have been obtained in an unaltered condition; experiments on single cells with the sap removed by micropipette might overcome this difficulty. A variety of other methods is available, but it would seem unlikely that the question can be settled decisively by any single one. The temperature dependence of the stationary state of a cell immersed in fluid might throw some light on the question, as would doubtless its dependence on respiratory poisons and oxygen tension (see some of the references in T. Levitt, 1947). Perhaps even the effect of varying such factors as the viscosity and conductivity of the plasmolyticum might be worth investigating.

Acknowledgement

It is a pleasure for the author to record his appreciation of the interest and criticism of Professor F. G. Gregory, with whom many of the points raised in this paper were discussed.

Appendix I

On Frictional or Contract Electrification

It seems strange that little attention seems to have been given to the transfer of electrons (as opposed to ions) as a source of the charges (i.e. the zeta-potentials) on colloidal particles and membranes. It is, of course, very well known that when two dissimilar substances are brought into contact a transfer of electricity almost always takes place, giving rise to a potential difference between them. In conductors the transfer affects the whole mass and produces the contact potential and Peltier effect; in non-conductors it is naturally restricted to the areas in actual contact and gives

rise to the phenomenon of static electrification. It is true that the nature of the process in the case of non-conductors is still a matter of controversy, but it seems likely that an inherent contact potential exists in this case too (N. K. Adam, 1941). Is it therefore impossible that electrification takes place at the oil-water interfaces of the plasma membrane producing charged water and lipoid molecules? And if such were the case, might not membrane potentials, coupled perhaps with active or passive water movements, carry the charged molecules away from the double layer in which they were formed to regions of higher positive or negative potential? If such indeed were the case we might expect interesting effects from a chemical standpoint, since the system would act like a minute galvanic battery deriving energy from osmotic or other sources, the electricity produced serving to discharge such ions as the hydrogen and hydroxyl. In accordance with Coehn's rule we should expect water to be positively electrified in contact with lipoids.

Even if the theory of contact electrification of insulators proves to be untrue, we still have the possibility that water molecules crossing the plasma membrane, perhaps in groups, might be *frictionally* electrified. The subject is interesting, but is beyond the scope of this paper.

Appendix II

On the Increase in Permeability caused by a Charged Membrane

For the sake of simplicity assume that the molecules of diffusing substance in the vicinity of the interface behave like those of a perfect gas. Then the distribution of their velocities in a direction perpendicular to the interface will be given by Maxwell's law:

$$\frac{1}{n}\,dn = \sqrt{\left(\frac{M}{2\pi RT}\right)}e^{-Mu^2/2RT}\,du, \tag{25}$$

where dn is the number of molecules with a velocity between u and $u+du$, n is the total number of molecules, M is the molecular weight, and R is the gas constant.

Assuming now that molecules with a speed u are able to cross the interface into the membrane, the rate at which they do so will be proportional to $u\,dn$, and the total rate at which molecules penetrate into the membrane will be given by

$$q = \int_{u=u_0}^{u=\infty} u\,dn = n\sqrt{\left(\frac{M}{2\pi RT}\right)}\int_{u_0}^{\infty} ue^{-Mu^2/2RT}\,du, \tag{26}$$

the integral being taken between u_0, the minimum velocity a molecule must have to overcome the potential energy barrier at the interface, and infinity.

The value of this integral works out as

$$q = n\sqrt{\left(\frac{RT}{2\pi M}\right)}e^{-Mu_0/2RT}, \tag{27}$$

which is, with minor adjustments, the value given by Davson and Danielli (1943). The term $Mu_0^2/2RT$ can be replaced by ϵ/kT, where k is Boltzmann's constant and ϵ is the height of the potential energy barrier per molecule.

Suppose now that by some mechanism—such as a membrane charge—the value of ϵ can be altered to ϵ'. Then q will become q', where

$$q' = n\sqrt{\left(\frac{RT}{2\pi M}\right)}e^{-\epsilon'/kT}.$$

Thus the ratio q'/q will be given by

$$q'/q = e^{(\epsilon-\epsilon')/kT}. \tag{28}$$

Consider now the case where a univalent ion in moving across the boundary traverses an electrostatic potential increment of 100 mV. Then the electronic charge being $4{\cdot}77\times10^{-10}$ e.s. units, we shall have

$$\begin{aligned}\epsilon-\epsilon' &= 4{\cdot}77\times10^{-10}\times(0{\cdot}1\times3{\cdot}34\times10^{-3})\text{ erg}\\ &= 1{\cdot}60\times10^{-13}\text{ erg.}\end{aligned}$$

Hence, at 20° C.,

$$\begin{aligned}\frac{(\epsilon-\epsilon')}{kT} &= \frac{1{\cdot}60\times10^{-13}}{1{\cdot}38\times10^{-16}\times293}\\ &= 4{\cdot}0.\end{aligned}$$

The 'permeability' of the interface will therefore be increased in the ratio $e^{4{\cdot}0}:1$, i.e. about 55 times. For a divalent ion, or for a step of 200 mV., the proportion would be $e^8:1$, i.e. 3,000 times and for a step of 400 mV. no less than 10^7 times. This increase of course relates only to the interface or to very thin membranes, and if the membrane is very thick and viscous its overall permeability may be hardly affected. It would seem, however, that with the plasma membrane this effect might be of very great importance. With the oppositely charged ion the permeability will of course be reduced, and in the same ratio.

Appendix III

On the Energy required for Active Transfer

An attempt has been made by J. Levitt (1947) to evaluate the possibility of active mechanisms being prominent in the water relations of the cell by a calculation of their energy requirements. In particular, he derived an expression relating the turgor pressure maintained actively to the rate of energy consumption. His argument runs somewhat as follows.

If a be the surface area of the cell, P the turgor pressure due to the active mechanism, and μ the permeability of the cell membrane to water, then under the influence of the turgor pressure alone water would pass out of the cell at the rate

$$Q = \mu aP. \tag{29}$$

Now imagine a mechanism simultaneously pumping water back at just this rate. The result will be a stationary state, and the rate of doing work of the mechanism will be

$$W = PQ = \mu aP^2, \tag{30}$$

μ being expressed as volume per unit time per unit pressure difference per unit area of membrane.

This formula clearly supposes that water is *actually* being circulated into and out of the cell; mere maintenance of turgor pressure without actual ordered movement

of some kind is an equilibrium condition requiring no expenditure of energy (cf. osmotically produced turgor). Supposing, however, that the formula is theoretically sound—a point which will be returned to later—then the author seems to have overestimated the area *a* in making his calculation. For if a number of similar cells be combined into a tissue and the whole immersed in a plasmolyticum, the appropriate area to use is surely not the total area of all the cell membranes, but rather the surface area of the tissue block, augmented perhaps by inter-cellular spaces. Levitt has used the former, and so instead of an area of about 100 sq. cm.[1] for a gramme of tissue he gets a value nearly $2\frac{1}{2}$ times as much.

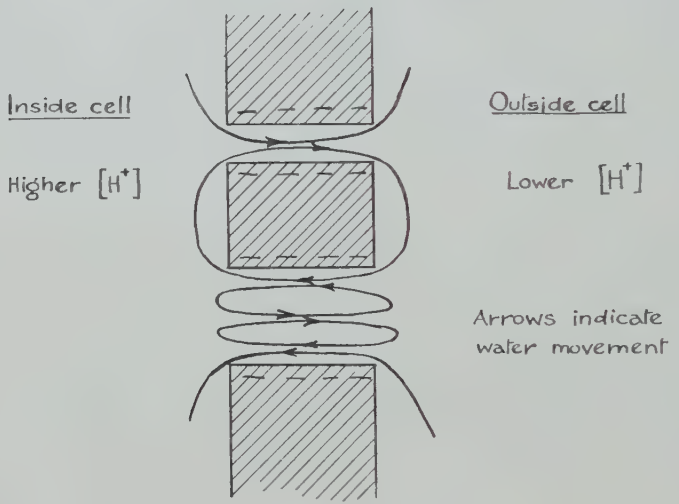


FIG. 10.

Strictly, too, it is the free energy change of glucose on oxidation that is important, not the heat of combustion, though in view of the fact that for carbohydrates the two are nearly identical it is quite likely that it was the former that was in mind and the latter used merely as an easily accessible approximation. Further, however, Bennet-Clark has questioned the suitability of Levitt's value for the permeability, and a recent paper (Myers, 1951) brings forward evidence that the permeability of the protoplast may be very much lower when it is in contact with the cellulose wall than when it is plasmolysed away from it—a condition analogous to which obtains in most measurements of permeability. Levitt calculates that the maximum rate of respiration observed by Bennet-Clark and his collaborators is only 2·5 times that required to supply the energy needed by the active mechanisms postulated by them. Surely that is enough? If the reply be made that it would involve the disappearance of the carbohydrate reserves of the organ at far too rapid a rate, the answer is that it is by no means necessary to assume that this high rate is operative in the *intact* organ; in fact oxygen diffusion would almost certainly set a limit to it. What Bennet-Clark and his colleagues believed they had measured was not the actively maintained suction pressure increment of massive organs but rather that of thin slices of tissue. In view of this there is little force in the argument

[1] Assuming that the tissue is in $\frac{1}{8}$ mm. slices.

that 'the beet would respire all of its dry matter in less than three months'—that might quite well be the case with thin slices if they kept their respiration up. Thus, even accepting Levitt's calculations as they stand, the respiratory energy of Bennet-Clark's samples is quite adequate to maintain a suction pressure increment of the order he found, and the force of this position is much increased when the criticisms of Levitt's numerical constants are added.

If, however, we examine the theoretical basis of equation (30), we find that it is hardly sound, that it represents a sort of limiting case, and that the energy expenditure actually involved may be anything down to zero. In fact an active mechanism can approximate 'asymptotically' to a passive one.

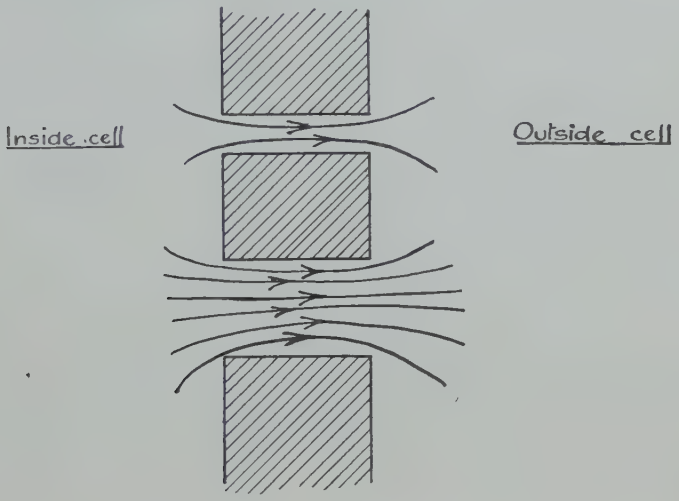


FIG. 11.

Consider, for instance, two pores in a cell membrane interacting electro-osmotically, and assume that the cell is fully turgid. Then there is no net flow of water inwards or outwards, and the actual movement of water that we are interested in will be that due to the turgor pressure superimposed on that caused by the electrical forces. The situation may be illustrated by reference to Fig. 10, where the electro-osmotic flow is inwards through the larger pores and outwards through the smaller ones, superimposed on it being an outward flow due to hydrostatic (turgor) pressure. If the electrical forces could be suddenly arrested the picture would become like that shown in Fig. 11, the immediate rate of working of the hydrostatic forces being given of course by equation (30). Now it should be at once obvious from an examination of these two systems of flow that the work being done in the two cases is not equal; of necessity, therefore, equation (30) cannot apply in the way intended.

How an 'active' agency can graduate into a 'passive' one can be illustrated by a simple model. Electro-osmosis is due to the frictional drag on the water of charged ions in a force field. Imagine it now to be replaced by a gravitational system, the pore becoming a vertical column of water and the ions falling particles of relative density greater than unity. If the particles are large they will fall with uniform velocity through the column, gravity doing work on the system and the 'drag' of the falling particles causing an enhanced pressure at the base of the column. If

now the particles be imagined to become smaller and smaller, their velocity will become less and less and with it the rate at which gravity does work on the system. Finally, when they are small enough they will remain permanently (though not uniformly) suspended, and no work will be involved at all. In all these cases, however, the increment of pressure at the base of the column due to the presence of the particles will be the same, but the 'active' mechanism with large falling particles showing organized movement has been replaced by a 'passive' one showing only random movement, the energy required to maintain the enhanced pressure falling from an undefined upper limit to zero. It might perhaps be added that 'permeability' measurements on the model would give the rate at which the water column would move if a pressure be applied at one end; what is wanted in order to calculate the energy expenditure is the rate at which the particles are falling—quite a different thing.

Exactly the same argument can be applied to 'active' mechanisms; as the ordered movements involved decline more and more to randomness, so the energy expenditure required to maintain the system falls; and a comparison between Figs. 10 and 11 would certainly suggest that such is the case here. Thus any attempt to calculate the energy requirement of an active mechanism must depend on a fairly detailed knowledge of the system concerned.

It might be asked how the transition from an active to a passive character could be imagined to take place in an electro-osmotic system. The answer is fairly clear. Starting with a membrane having a low zeta-potential and bathed by a dilute salt solution, imagine the zeta-potential to be increased till ions of one sign only can pass. Further, let the salt solution be increased[1] in strength till all the water molecules are bound in the ionic shells. Then we have reduced the system from a state in which electro-osmosis is a very inefficient sustainer of the turgor pressure (its upper energy requirement being quite undefinable) to a state in which like osmosis it requires no energy at all to do so.

Literature Cited

Adam, N. K., 1941: The Physics and Chemistry of Surfaces. Oxford.

Bennet Clark, T. A., Greenwood, A. D., and Barker, J. W., 1936: Water Relations and Osmotic Pressures of Plant Cells. New Phyt., xxxv. 277–91.

—— and Bexon, D., 1940: The Water Relations of Plant Cells, II. Ibid., xxxix. 337–61.

—— —— 1946: Water Relations of Plant Cells, IV. Diffusion Effects observed in Plasmolysed Tissues. Ibid., xlv. 1–17.

——, 1948: Interaction of Water and Porous Materials. A discussion of the Faraday Society.

Crafts, A. S., Currier, H. B., and Stocking, C. R., 1949: Water in the Physiology of Plants. Waltham, Mass.

Curtis, O. F., 1937: Vapour Pressure Gradients, Water Distribution in Fruits, and So-called Infra-red Injury. Amer. Journ. Bot., xxiv. 705–10.

Davson, H., and Danielli, J. F., 1943: The Permeability of Natural Membranes. Cambridge.

Freundlich, H., 1926: Colloid and Capillary Chemistry. London.

Höber, R., 1945: The Physical Chemistry of Cells and Tissues. New York and London.

Levitt, J., 1947: The Thermodynamics of Active (Non-Osmotic) Water Absorption. Plant Phys., xxii. 514–25.

Lewis, W. C. McC., 1909: An Experimental Investigation of Gibbs's Theory of Surface Concentration. Phil. Mag., xvii. 466–94.

Meyer, B. S., 1945: A Critical Evaluation of the Terminology of Diffusion Phenomena. Plant Phys., xx. 142–64.

[1] These two conditions, of course, could hardly be found together in actual systems.

Myers, G. M. P., 1951: The Water Permeability of Unplasmolysed Tissues. Journ. Expt. Bot. ii. 129–44.

Oppenheimer, H. R., 1930: Ber. deutsch. bot. Ges., xlviii. 130–40.

Osterhout, W. J. V., and Murray, J. W., 1939: Behaviour of Water in Certain Heterogenous Systems. Journ. Gen. Phys., xxiii. 365–90.

Teorell, T., 1938: Studies in the Diffusion Effect upon Ionic Distribution, II. Ibid., xxi. 107–22.

Thoday, D., 1950: On the Water Relations of Plant Cells. Ann. Bot., n.s., xiv. 1–6.

Van den Honert, T. H., 1932: On the Mechanism of the Transport of Organic Materials in Plants. Konink. Akad. Wetensch. Amsterdam Proc., xxxv. 1104–12.

The Germination of the Seeds of *Rhinanthus Crista-galli*

BY

K. B. VALLANCE

(*Hartley Botanical Laboratories, University of Liverpool*)

With four Figures in the Text

Abstract

The germination of the seeds of *Rhinanthus Crista-galli* has been induced by exposing them to moisture at 2° C. for periods of from 17 weeks to over a year, depending on the amount of dry storage to which the seeds had previously been subjected. Germination could not be brought about by moisture-treatment at 20° C. During moisture-treatment at 2° C. the respiration rate falls significantly after an initial increase, and then gradually rises, after which germination takes place. Generally, respiration is significantly lower during moisture-treatment at 20° C. Analyses of the treated seeds suggest that the respiratory substrate might be protein. It was found by paper chromatography that during moisture-treatment at 2° C. the number of amino-acids in the alcohol extract first diminishes and then increases, reaching a maximum in the ungerminated seed just before germination becomes apparent. Differences were detected in the amino-acids found in the alcohol extracts of seeds treated at 2° C. compared with those moisture-treated at 20° C. Reasons are given which suggest that the limiting factor in the germination process may be the nature and rate of the hydrolysis of the reserve proteins of the seeds.

Introduction

THE work on *Rhinanthus* as originally planned was not concerned directly with the germination of the seeds but with the growth requirements of the seedlings. However, attempts to germinate the seeds by sowing on damp filter-paper at room temperature failed; in fact no germination was induced by even a year of such treatment. It was then found that Heinricher (1909) had discovered that seeds of *Rhinanthus* spp. sown in the autumn did not germinate until the following spring, and furthermore that he had thought it possible that there might be some correlation between the degree and duration of the winter coldness and germination. This work of Heinricher's suggested that germination might be brought about in the laboratory by means of low-temperature stratification. Initial experiments showed that cold moist treatment would induce germination, but even under optimum conditions germination did not take place for some weeks. In consequence it was thought that it would be a matter of some interest to investigate the germination process in some detail before studying the growth requirements of the seedlings. The present paper concerns the effect of cold treatment on seed germination, on seed respiration, and on the major storage materials of the seeds.

[Annals of Botany, N.S. Vol. XVI, No. 63, July, 1952.]

The seeds of *Rhinanthus Crista-galli* employed were gathered from behind the sand-dunes at Lytham St. Annes in August 1948 and 1949, and from the dune slacks at Freshfield in June and July 1950.

Experimental Methods

When germination alone was being determined, seeds of *Rhinanthus* were placed inside 12 sintered crucibles, so that the seeds just covered the sintered discs. Each crucible and its contained seeds was then placed inside a 150-ml. Pyrex beaker and the beaker was filled with distilled water up to the lower level of the sintered disc. The beakers were then covered with Petri-dish lids. Half the beakers of each series were then placed in a refrigerator carefully regulated so as to maintain a temperature of 2° C., whilst the other half were maintained at 20° C. and thus served as controls. Each beaker was examined once per week and germinated seeds, if present, were removed.

When seeds were required for chemical analysis and the measurement of respiration as well as for germination tests, 2 g. of seeds were added to each sintered crucible. In these cases care was taken to maintain the water at its initial level during the period of imbibition. Seeds from these latter crucibles were also used to determine the effect on germination of raising the temperature from 2° C. to 15° C. For this determination 25 seeds were removed from each of 6 sintered crucibles, and each seed lot was placed in a small Petri-dish on a pad of damp filter-paper. These Petri-dishes were examined for signs of germination twice per week.

The respiration of *Rhinanthus* seeds was determined in Barcroft respirometers, the thermostatically controlled bath for which was maintained at 22° C. Results are expressed as μl. of gas at N.T.P. per mg. dry weight per hour and each point plotted in the figures is the mean of three observations. All results were analysed for variance.

Some indication of the nature of the seed sugars at certain stages in the germination process was obtained from paper chromatograms, using similar methods to those of Partridge (1949 *a* and *b*). Control runs with a sucrose-glucose mixture before and after acid hydrolysis at the same time gave spots in the same positions and of the same colour as the seed sugars, and so it was considered possible that the seed sugars consisted of glucose and sucrose. Quantitative estimations of the amount of sugar present were therefore made on this assumption, using the method of Weinmann (1944, 1947).

No analysis of the seed fat was attempted. The figures given are those of the weight of the ether-soluble material of the seeds.

The total nitrogen of the dried, freshly gathered seeds and of the dried, germinated seeds was determined by the Kjeldahl method, using the digestion procedure of Chibnall, Rees, and Williams (1943), the distillation apparatus of Markham (1942), and the distillation procedure of Ma and Zuazaga (1943).

The total amino-acid content of the freshly gathered and the germinated seeds together with the amino-acid composition of the alcohol-soluble nitrogenous material of the seeds at various stages in the germination process was

qualitatively determined by means of two-dimensional chromatograms. The material for analysis was prepared in a similar way to that used by Allsop (1948), except that it was always found necessary to hydrolyse the alcoholic extract as otherwise no amino-acids could be identified. The two-dimensional chromatograms were prepared in the way recommended by Dent (1948).

Experimental Results

Germination

Germination tests were first carried out in April 1949, using seeds harvested in the previous year. During the period between the harvesting of the seed and the beginning of the germination tests the seeds were stored in a stoppered glass bottle in the laboratory. In these first tests 10 per cent. of the seeds germinated after 4 weeks of moisture-treatment at 2° C. followed by 14 days at the enhanced temperature of 15° C. Germination was also observed amongst those seeds still in the refrigerator after 8 weeks of cold treatment. After 12 weeks of cold treatment this germination inside the refrigerator had reached 25 per cent., but no subsequent germination took place.

Further experiments were initiated in January 1950, using seeds harvested in the previous year. After 7 weeks of moisture-treatment 20 per cent. of the seeds were induced to germinate by raising the temperature from 2° C. to 15° C. for 14 days. After 9 weeks the germination obtained in this way reached 25 per cent., but from this stage onwards raising the temperature was less effective in promoting germination, and after 19 weeks of moisture-treatment at 2° C. no further germination could be induced by raising the temperature. In these experiments with 1949 seeds, germination inside the refrigerator first became apparent after 11 weeks of moisture-treatment, and sporadic germination continued for more than one year. This may be seen from Fig. 1. As expected from the work of Heinricher (1909), those viable seeds which had been first exposed to moisture at 2° C., and then to moisture at 15° C. without germinating, could be induced to germinate by further treatment at 2° C.

These experiments on 1949 seeds further indicated that dry storage might reduce the viability of the seeds and hence it seemed desirable to expose seeds to moisture-treatment immediately after harvesting. This was done with seeds harvested in 1950, and the resultant germination inside the refrigerator may also be seen from Fig. 1. This figure shows that no germination was apparent after 14 weeks of moisture-treatment, but that a mean germination percentage of 95 per cent. was obtained after 17 weeks. An experiment initiated 5 weeks later in which the same seeds were similarly treated gave different results. In this latter experiment the effect of raising the temperature to 15° C. after varying degrees of moisture-treatment was determined. Results similar to those obtained are given in Fig. 3. This figure shows that in common with the previous data germination could not be induced by raising the temperature of moisture-treatment from 2° C. to 15° C. until 3–4 weeks before germination became apparent in the refrigerator.

A further germination test on 1950 seeds was carried out in order to determine whether or not an initial period of moisture-treatment at 20° C. would reduce the time required for subsequent germination to take place during moisture-treatment at 2° C. In this test 1950 seeds were exposed to moisture at 20° C. immediately after harvesting. After 5 months of this treatment they were transferred to the refrigerator. As in the series of 1950 seeds in which the seeds were immediately exposed to moisture at 2° C., no

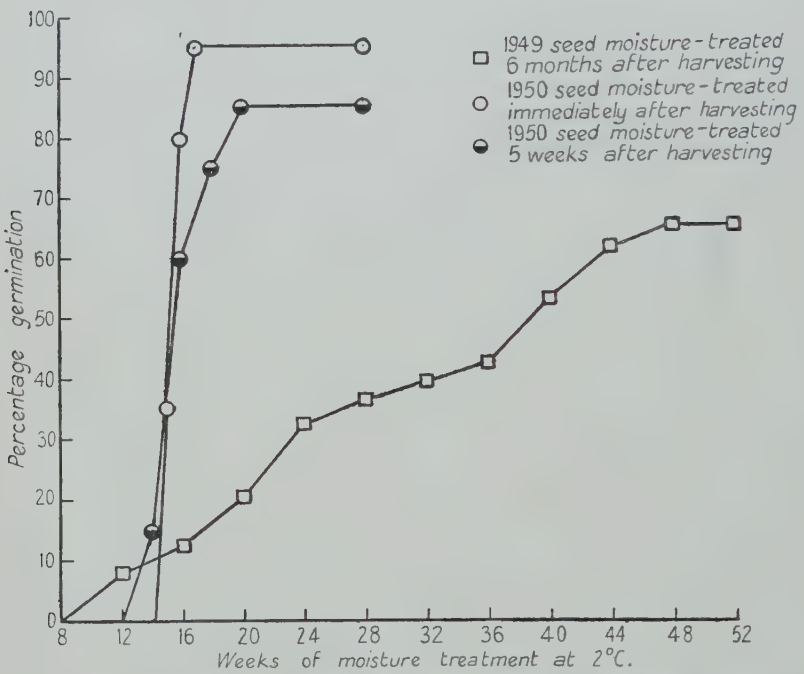


FIG. 1. The rate of germination of the seeds of *Rhinanthus Crista-galli* during moisture-treatment at 2° C.

germination was visible after 14 weeks of moisture-treatment at 2° C. However, after 15 weeks 65 per cent. of the seeds had germinated in contrast to 36 per cent. in the earlier series, and after 16 weeks 95 per cent. had germinated compared with 80 per cent. in the original experiment. Hence the only effect of the earlier treatment at 20° C. was to shorten by 1 week the period within which germination actually took place, that is, from 3 weeks to 2 weeks.

Respiration and the gross chemical changes during moisture-treatment

The rate of respiration of the newly germinated seeds was found to be much higher than that of seeds at any other stage of the moisture-treatment process. Studies of the drift of respiration of 1949 and 1950 seeds during their moisture-treatment at 2° C. showed that this high level of respiration was not evident until from 2 to 3 days before the radicle became apparent. Apart from the

changes in the rate of respiration immediately prior to germination, the drift of respiration at 2° C. of both the 1949 and 1950 seeds followed a very definite pattern, though certain differences were found, and so both series are given in the present paper. The data of an experiment in which 1950 seeds were exposed to moisture at 20° C. are also given.

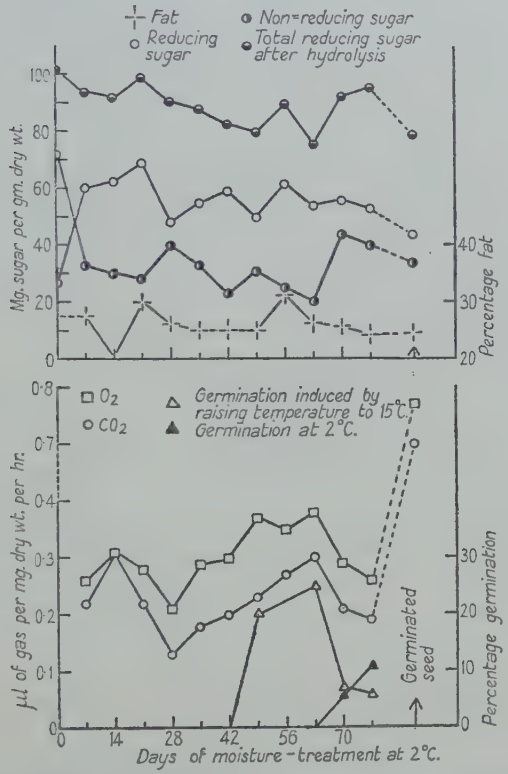


FIG. 2. The drift of respiration, the amount of germination, and the changes in the seed fat and sugars during moisture-treatment of 1949 seed at 2° C. (Standard error of respiration data = ±0·027, that of sugar data = ±4·7.)

1949 seed. Moisture-treatment at 2° C.

Fig. 2 shows the drift of respiration, some germination data, and certain information on the changes in seed fat and sugar during moisture-treatment at 2° C. It may be seen from this figure that the rate of respiration initially increased and reached a peak value at the 14th day. After this the rate of respiration diminished until the 28th day. This fall in the respiratory rate was followed by a gradual rise up to the 63rd day. It may be of interest to note that germination induced by increasing the temperature from 2° C. to 15° C. could not be brought about until the carbon dioxide level had risen significantly from the 28th-day level. The figure also shows that after the

63rd day, when the rate of respiration fell once more, the amount of germination induced by raising the temperature to 15° C. was also diminished.

A study of the changes in the fat and sugar content of the seeds gave little indication of the metabolic changes leading to germination. Thus between 42 and 63 days, when some germination was induced by raising the temperature of moisture-treatment, the changes in non-reducing sugar and reducing sugar were not significant. However, during the period from 63 to 70 days, when germination inside the refrigerator first became apparent, the non-reducing and total sugar content increased significantly.

The nature of the respiratory substrate utilized during the respiration of the 1949 seed is not clear from the data available. On the conventional view the R.Q.s, as may be seen from the figure, would suggest that this substrate was largely fat. However, some germinated seeds obtained over 6 months after the beginning of the experiment contained 23 per cent. (mean of 2 determinations) of crude fat. This represents a fall of some 4·6 per cent. of fat, and in consequence it would scarcely seem probable that much fat was respired during the 6-month period.

1950 seed. Moisture-treatment at 2° C. and 20° C.

Fig. 3 shows some of the data obtained during the moisture-treatment of a sample of 1950 seed at a temperature of 2° C. From this figure it may be seen that the changes in the drift of respiration of the 1950 seeds were by no means so marked as in the case of the 1949 seeds. Nevertheless, after the initial rise from the level of the dry seed the rate of O_2 input and CO_2 output fell significantly during the first part of the experiment. Furthermore, from 63 days onwards, when it became possible to germinate some seeds by raising the temperature, the level of oxygen input was significantly higher than at the 42nd-day level. The final rise in respiratory intensity, from 84 to 91 days, would seem to be due to the onset of germination of the majority of the seeds.

As in the case of the 1949 seeds, the changes in the fat and sugar content gave little indication of the metabolic changes leading to germination. However, the general level of non-reducing sugar tended to be higher in the 1950 seeds, in which dormancy was not so pronounced, than in the 1949 seed. Again, in a similar way to the 1949 seeds, the reducing sugar content first increased and then diminished, due largely apparently to the interconversion of reducing and of non-reducing sugar and vice versa. At the same time as the reducing sugar content reached its lowest value (during the first phase) the level of oxygen input and carbon dioxide output was also at its lowest.

The variation in the crude fat content of the 1950 seeds was less than that in the case of the 1949 seeds and even the newly germinated seeds contained some 27 per cent. of crude fat.

The data relating to the moisture-treatment of the 1950 seeds at 20° C. are given in Fig. 4. From this figure it may be seen that the most obvious difference between the effect of moisture-treatment at 20° C. and at 2° C. was in respect to the respiratory intensity. Thus at the higher temperature the rate

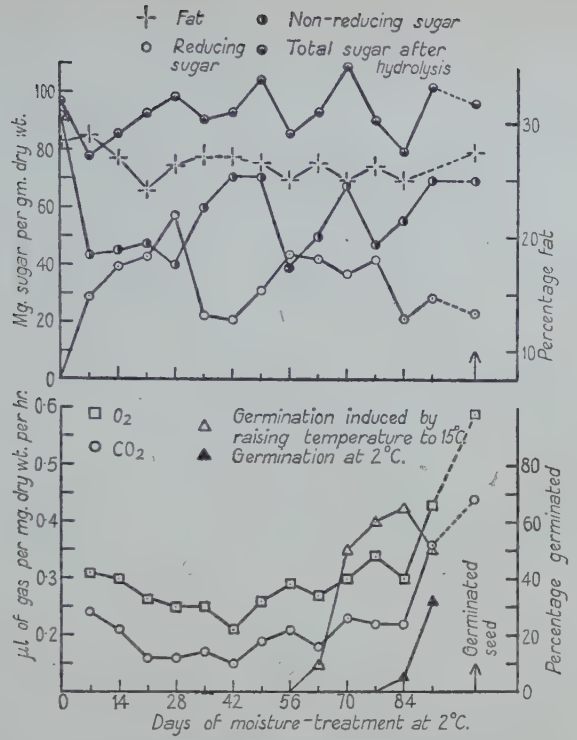


FIG. 3. The drift of respiration, the amount of germination, and the changes in the seed fat and sugars during 13 weeks of moisture-treatment of 1950 seed at 2° C. (Standard error of respiration data = ±0·02, that of sugar data = ±4·3.)

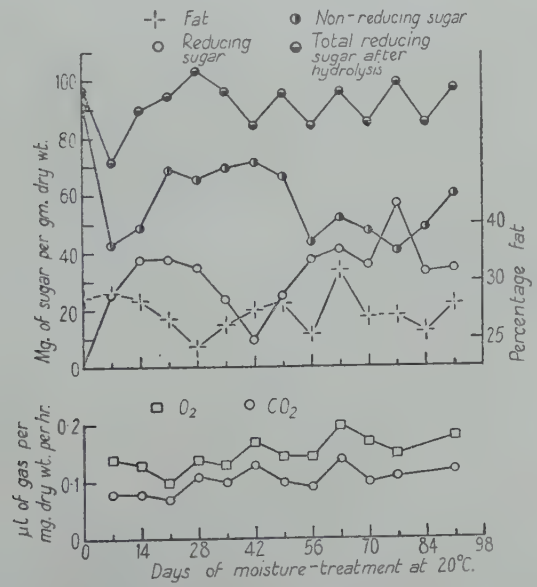


FIG. 4. The effect of moisture-treatment at 20° C. on the respiration and the fat and sugar content of 1950 seeds. (Standard error of respiration data = ±0·02, that of sugar data = ±4·2.)

of respiration over the 7– to 91-day period was generally significantly lower. This effect of the higher temperature on respiration would in itself seem to be sufficient to account for the absence of germination at 20° C. Strangely enough, the sugar and fat curves of Figs. 3 and 4 are surprisingly similar, and hence except for the effect of the higher temperature on respiration it would seem that the data of Figs. 3 and 4 do not explain the occurrence or otherwise of germination.

The changes in the alcohol-soluble nitrogenous material of the seed during moisture-treatment

As a first step in the investigation of these changes two-dimensional chromatograms were prepared of some of the material of the total hydrolysates of germinated and non-treated seeds in order to find out whether any changes took place in the nature of the amino-acids present in the total nitrogenous material during the germination process. Each determination was replicated once. The method employed would not show tryptophane or cystine, but otherwise the same amino-acids were present in both germinated and non-treated seeds. These amino-acids were provisionally identified as α-amino *n*-butyric acid, γ-amino butyric acid, tyrosine, lysine, arginine, phenylalanine, proline, threonine, serine, α-alanine, glycine, isoleucine or leucine, valine, aspartic acid, and glutamic acid. Further observations showed that these same amino-acids were present in both the embryo and the endosperm of the germinated seed.

Table I shows the changes in the amino-acid composition of the alcohol extract of the seeds during certain stages of moisture-treatment at 2° C., and from the table it may be seen that within the experimental limits the number of amino-acids in the alcohol extract diminished progressively during the first 7 weeks. Thus after 3 weeks arginine was missing. After 6 weeks arginine was missing from both series, alanine, serine, and glycine were missing from one, and the latter two were only represented by very faint spots in the second series. After 7 weeks only leucine (or isoleucine), valine, aspartic acid, and glutamic acid could be detected. A further point of interest was noted in the 6th-week chromatograms. This was that the areas of the spots, as determined by a planimeter, were smaller than those at any other stage of moisture-treatment which was investigated. After 7 weeks there was an increase in the number of amino-acids in the alcohol extract, and at the 13-weeks' stage, when germination in the refrigerator was first observed, the number of amino-acids present in both series had increased to phenylalanine, proline, α-alanine, glycine, serine, arginine, leucine (or isoleucine), valine, aspartic acid, and glutamic acid, and lysine was detected in one series. The germinated seeds differed from this last series in that proline and phenylalanine were absent and threonine was present.

Table I suggests strongly that the limiting factor in the germination process may be the number of amino-acids available in peptide form. It seems possible that the rate of respiration of moisture-treated *Rhinanthus* seeds may be

controlled by the number and amount of the amino-acids available in the soluble nitrogenous fraction. Thus, for example, as may be seen from Fig. 3, after 6 weeks the rate of respiration was at its lowest level, and at 13 weeks at its highest, before germination became apparent.

TABLE I

The Amino-acid Composition of Alcohol Extracts of 1950 Seeds during Moisture-treatment at 2° C.

	Days of moisture-treatment at 2° C.									Germinated seed.
Amino-acids.	0	21	42	49	56	63	77	84	91	
α-Amino *n*-butyric acid										
γ-Amino butyric acid						+				
						+				
Tyrosine										
Lysine										+
					Faint				+	+
Phenylalanine									+	
									+	
Proline									+	
									+	
Threonine					Faint			Faint		+
					Faint					+
Arginine	+				Faint		+	+	+	+
	+				Faint	+		+	+	+
Serine	+	+			+	Faint	+	+	+	+
	+	+	Faint		+	Faint	+	+	+	+
α-Alanine	+	+			+	+	+	+	+	+
	+	+	+		+	+	+	+	+	+
Glycine	+	+			+		+	+	+	+
	+	+	Faint		+	+		+	+	+
Leucine or Isoleucine	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+
Valine	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+
Aspartic acid	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+
Glutamic acid	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+

This supposition is further supported by the data concerning moisture-treatment at 20° C. which are given in Table II. Threonine, proline, arginine, and lysine were either absent from the alcohol-soluble material or else only represented by very faint spots. Furthermore, well-defined spots of phenylalanine were only found on the chromatograms prepared from the 13-week material.

TABLE II

The Amino-acid Composition of Alcohol Extracts of 1950 Seeds during Moisture-treatment at 20° C.

Amino acids.	Days of moisture-treatment at 20° C.							
	0	21	42	56	63	77	84	91
α-Amino *n*-butyric acid								
γ-Amino butyric acid								
Tyrosine								
Lysine								
Phenylalanine			Faint					+
			Faint					+
Proline								
Threonine				Faint				
Arginine	+				Faint		Faint	
	+				Faint		Faint	
Serine	+	+		+	Faint	Faint	+	+
	+	Faint	Faint	+	Faint	+	+	+
α-Alanine	+	Faint		+		+	+	+
	+	+	+	+		+	+	+
Glycine	+	Faint		+	+	+	+	+
	+	Faint		+	Faint	+	+	+
Leucine or Isoleucine	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+
Valine	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+
Aspartic acid	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+
Glutamic acid	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+

The evidence thus suggests that the respiration and germination of *Rhinanthus* seeds may depend in some way on the available alcohol-soluble nitrogenous material. This view is not in disagreement with the views of Gregory and Sen (1937). It is supported by the data of Table III, which shows the amounts of the major seed reserves present in the non-treated and newly germinated seeds. The high protein content is noticeable. The possibility also exists, however, that the respiration was a fat respiration, in which the loss in weight of the fat was largely compensated for by oxidation. On the whole, however, the data in this paper agree rather better with the view that the controlling factor in the germination process may be the rate and nature of the hydrolysis of the reserve proteins.

TABLE III

The Reserve Materials of Non-treated and Germinated Seeds expressed as Percentages of the Dry Weight

	Non-treated seeds.	Germinated seeds.
Mean weight of one dried seed	0·00241 g.	0·00256 g.
Testa	19·4%	19·4%
Ash	3·4%	—
Total 'N'	4·42%	4·48%
Polysaccharide	13·8%	15·5%
Non-reducing sugar	9·2%	7·0%
Reducing sugar	0·0%	2·3%
Crude fat	28·6%	27·37%

Results for polysaccharide, reducing and non-reducing sugar are the mean values obtained from 2 series each of 6 replicates. Results for testa, ash, and nitrogen are the means of 6 determinations, fat the mean of 2 determinations, and the mean weight of a seed was calculated from the weights of 6 lots of 40 seeds in each case.

Furthermore, the absence of certain amino-acids from the alcohol extract of seeds treated at 20° C., compared with those treated at 2° C., suggests that protein hydrolysis might be retarded at the higher temperature. Such a retardation might account for both the low rate of respiration and for the absence of germination of seeds treated at 20° C.

SUMMARY

1. The germination of the seeds of *Rhinanthus Crista-galli* has been induced by exposing them to moisture at 2° C. Raising the temperature of moisture-treatment from 2° C. to 15° C. was found to promote germination before it became apparent at 2° C., but only during the 3–4 weeks before the first germination occurred at 2° C.

2. Studies of the respiration of 1949 and 1950 seeds during moisture-treatment at 2° C. showed that after the initial increase in the rate from that of the dry seeds the intensity of respiration significantly diminished, as measured by O_2 input or CO_2 output. This fall was followed by a further rise in the respiratory rate. Germination could not be induced until this latter phase had been attained. Moisture-treatment at 20° C. significantly depressed the respiration, compared with that at 2° C.

3. Analysis of the seeds showed that the principal reserves were fat, sugar, and protein. During moisture-treatment for periods of up to 13 weeks the loss in fat and sugar was not such as would suggest that these two reserves were being respired. It was thus concluded that the seed respiration during moisture-treatment was largely a protein respiration.

4. Using paper chromatograms, studies were made of the amino-acids of the alcohol-soluble nitrogenous material of seeds exposed to moisture at 2° C.

and 20° C. During moisture-treatment at 2° C. the number of amino-acids in the alcohol extract first diminished and then increased, reaching a maximum just before germination became apparent. Certain of the amino-acids found in the alcohol-soluble material of seeds moisture-treated at 2° C. were not present in the alcohol extract of seeds exposed to moisture at 20° C.

5. The data as a whole suggested that the controlling factor in the germination process might be the nature and rate of the hydrolysis of the reserve proteins of the seeds.

In a private communication the author has been informed by Dr. P. E. Stokes, of the Botany Department, University College, London, that there are certain similarities between the germination process of the seeds of *Heracleum Spondylium* and that of the seeds of *Rhinanthus Crista-galli.*

Literature Cited

Allsop, A., 1948: Chromatographical Study of Meristematic Plant Tissues. Nature, clxi. 833.

Chibnall, A. C., Rees, M. W., and Williams, E. F., 1943: The Total Nitrogen Content of Egg Albumin and Other Proteins. Biochem. Journ., xxxvii. 354.

Dent, C. E., 1948: A Study of the Behaviour of some Sixty Amino-acids and other Ninhydrin-reacting Substances on Phenol-collidine Filter Paper Chromatograms, with Notes on the Occurrence of some of them in Biological Fluids. Ibid., xliii. 169.

Gregory, F. G., and Sen, P. K., 1937: Physiological Studies in Plant Nutrition, VI. Ann. Bot., n.s., i. 521.

Heinricher, E., 1909: Die grünen Halbschmarotzer, V. Jahrb. f. wiss. Bot., xlvi. 273.

Ma, T. A., and Zuazaga, G., 1942: Micro-Kjeldahl Determination of Nitrogen. A New Indicator and an Improved Rapid Method. Industr. Engng. Chem. (Anal. ed.), xiv. 280.

Markham, R., 1942: A Steam Distillation Apparatus suitable for Micro-Kjeldahl Analyses. Biochem. Journ., xxxvi. 790.

Partridge, S. M., 1949 *a*: Partition Chromatography and its Application to Carbohydrate Studies. Biochemical Society Symposia, iii. 52.

—— 1949 *b*: Analine Hydrogen Phthalate as a Spraying Reagent for Chromatography of Sugars. Nature, clxiv. 443.

Weinmann, H., 1944: Semi-micro Estimation of Reducing Sugars. Plant Physiol., xix. 148.

—— 1947: Determination of Total Available Carbohydrate in Plants. Ibid., xxii. 279.

The Diatom Genus Tabellaria

I. Taxonomy and Morphology

BY

BRENDA M. KNUDSON

(*Freshwater Biological Association, Ambleside, Westmorland, England*)

With seven Figures in the Text

ABSTRACT

A morphological study of natural populations of *Tabellaria* in fresh water suggested that there were two fundamentally different types of growth (and therefore frustule structure) in the genus, and this has been confirmed by means of unialgal clone cultures. The existence of these different types of growth forms the basis for a revision of the genus. A diagnosis of *T. quadriseptata* nov. spec. is given, the specific limits of *T. fenestrata* and *T. flocculosa* are redefined, and the occurrence of *T. binalis* in Britain is reported for the first time.

I. INTRODUCTION

THE genus *Tabellaria* is commonly believed to consist of three species: *T. fenestrata* (Lyngb.) Kütz., *T. flocculosa* (Roth) Kütz., and *T. binalis* (Ehr.) Grun. in V.H. *T. binalis* is so distinctive morphologically (see Fig. 7) that it cannot be mistaken for any other member of the genus, and it will not be mentioned again except in the conspectus. Doubts on the separate identity of *T. fenestrata* and *T. flocculosa* were expressed by Bachmann (1907, pp. 67–70), who regarded these two species as the end members of a single morphological series, linked by *T. fenestrata* var. *intermedia* Grun. in V.H. Positive evidence for regarding *T. fenestrata* and *T. flocculosa* as distinct species has been obtained by three main methods, viz. by studying (1) the taxonomic history of the genus; (2) the range of structure in nature as shown by collections from many different localities and habitats; and (3) the changes undergone by the two species when grown in unialgal clone culture.

The following sub-sections give elementary accounts of the morphology of these species and of the present taxonomic criteria used in their determination.

Morphology. In *Tabellaria*, the cells are united into colonies whose shape and structure provide characters of diagnostic importance (see below). Between valve and connecting band of each semi-cell lie one or more thin intercalary bands each of which supports a siliceous septum (Fig. 6, H *s*). Septa are formed at alternate poles of adjacent intercalary bands. In some specimens of *T. flocculosa* septa develop at both poles of the intercalary bands, those at one pole being very short. In girdle view they may be very prominent (Fig. 2, 1). On boiling with acid the intercalary bands fall apart, and the two

[Annals of Botany, N.S. Vol. XVI, No. 63, July, 1952.]

unequally developed septa may then be seen in a single intercalary band (Fig. 5, F, G). This phenomenon has only been observed in *T. flocculosa*, and it is not invariably present in that species. In *T. fenestrata* only one pole of the intercalary band becomes occluded. In mature frustules of *T. quadriseptata* minute dots or flanges are sometimes seen in girdle view between the septa. These are not so regularly arranged as in *T. flocculosa*, and some of them may be the precursors of septa which reach full development only when the cell next undergoes division. All such structures, whatever their nature, are termed 'rudimentary septa' in this paper. The valve is linear except for central and apical inflations, and bears numerous transapical striae separated by a narrow pseudoraphe (Fig. 1, B *ps*). Since the length of the apical axis varies relatively more than the other axes, the measurement of valve length provides the most convenient guide to the size of a *Tabellaria* population. In this paper the term 'length range' refers to the extreme measurements of valves measured along the apical axis.

When cell-division takes place, the girdle region of the cell widens along the pervalvar axis and the new valves are formed (Fig. 6, G *n.v.*). In *T. fenestrata* and *T. quadriseptata* four septa are present when this happens; consequently each newly formed daughter-cell has two septa. In *T. flocculosa* the number of septa at the time of cell-division is very variable (see p. 426). New intercalary bands and septa form between the new valve and its connecting band; in *T. flocculosa* (and probably in the other species too) the intercalary band nearest the girdle is the youngest.

After division the two daughter-cells do not separate. They are joined at one apex by mucilage secreted by mucilage pores which are very difficult to observe. A chain colony is formed if the daughter-cells separate sometimes at one pole, sometimes at the other. In *T. fenestrata* the mature cells lie at an angle of 180° to each other, thus giving the appearance of a straight filament (Fig. 1, A); whereas in *T. flocculosa* (Fig. 5, D, E) and *T. quadriseptata* (Fig. 1, D, E) the angle between mature cells may be acute, right, or obtuse. If the cells of successive divisions separate at the same pole, a star type of colony results (*T. fenestrata* var. *asterionelloides*, Fig. 4, B), but the two types of separation may sometimes be seen in a single colony (Fig. 4, A).

Classification. The following characters, summarized from Hustedt (1931, pp. 26–30), are those normally given for the identification of *T. flocculosa*, *T. fenestrata*, and the three chief varieties which have been ascribed to *T. fenestrata*, viz. var. *intermedia* Grun. in V.H., var. *asterionelloides* Grun. in V.H., and var. *geniculata* A. Cleve.

T. fenestrata: resting cell with four intercalary bands and septa. Valve 30–140 μ long, 3–9 μ broad; central swelling scarcely broader than apical inflations.
var. *intermedia*: cell with more than four intercalary bands.
var. *asterionelloides*: cells forming star-shaped colonies, but often changing into zigzag chains.
var. *geniculata*: cells bent in the middle, mostly forming star-shaped colonies.
T. flocculosa: cell with numerous intercalary bands and septa. Valve 12–50 μ

long, 5–16 μ broad; central swelling generally considerably broader than the apical inflations.

II. Taxonomy of *T. fenestrata* and *T. flocculosa*

Under this heading will be described (1) the taxonomic history of these species and the varieties known as *T. fenestrata* var. *intermedia* and *T. fenestrata* var. *asterionelloides*; (2) the experimental evidence for considering *T. fenestrata* and *T. flocculosa* as distinct species. The remaining taxonomic units (taxa), on which no experimental work has yet been done, are discussed in a later section on the specific limits of *T. flocculosa*.

1. *History of the main taxa*

Tabellaria fenestrata (Lyngbye) Kützing

This species was first described as *Diatoma fenestratum* by Lyngbye (1819). His drawing (pl. 61, fig. 3) and the type material, preserved in the Universitetets botaniske Museum, Copenhagen, show that the cells are joined to form 'straight-line' colonies (cf. Fig. 1, A). By 'the longitudinal line near each edge' Lyngbye was obviously referring to the septa, which he was unable to distinguish as separate entities. Cells with 2, 3, and 4 septa are found in the type material. One of the diagnostic characters which Lyngbye gave, viz. a band of 3 round granules, must have been due to bad preservation of the material and its nature is unknown. The type material of *T. fenestrata* is mixed with *T. flocculosa* but the length ranges do not overlap (*T. fenestrata* 49–73 μ; *T. flocculosa* 15–45 μ). The cell proportions in the drawing show conclusively which species was meant, and as will be shown later, the 'straight-line' type of colony morphology is peculiar to *T. fenestrata*. The type material of *T. fenestrata* has the following additional characteristics:

(1) In valve view the apical inflations are distinctly capitate and are approximately equal in width to the central inflation.
(2) Each septum has a sharp bend very close to its point of insertion so that this is farther from the valve than its free end.
(3) Absence of rudimentary septa.
(4) Eighteen striae in 10 μ.
(5) Division of the cell takes place when there are four septa, so that daughter-cells contain two septa apiece at first.
(6) A prominent mucilage pore on the central inflation of the valve, the pore being situated near the centre of the inflation.

Several varieties have been described under this specific name, but, as is pointed out below, they do not belong to this species. Accordingly, wherever the name *T. fenestrata* without any added varietal epithet is used in this paper it refers to diatoms agreeing with Lyngbye's specimens as described above.

One of the most commonly quoted descriptions purporting to be of *T. fenestrata* is Smith's (1856, vol. ii, p. 46). Smith emphasizes the 'definite' structure of the frustule, by which he means that the maximum number of

septa per cell is four. His illustrations (pl. 43, fig. 317) include four colonies labelled *T. fenestrata*. Only one of these (317 f[III]) shows any resemblance in shape and valve structure (capitate apical inflations) to *T. fenestrata*. The other three show zigzag *Tabellaria* colonies. The girdle views of these show that the specimens were neither *T. fenestrata* nor *T. flocculosa*. This is confirmed by reference to Smith's specimens in the British Museum (Natural

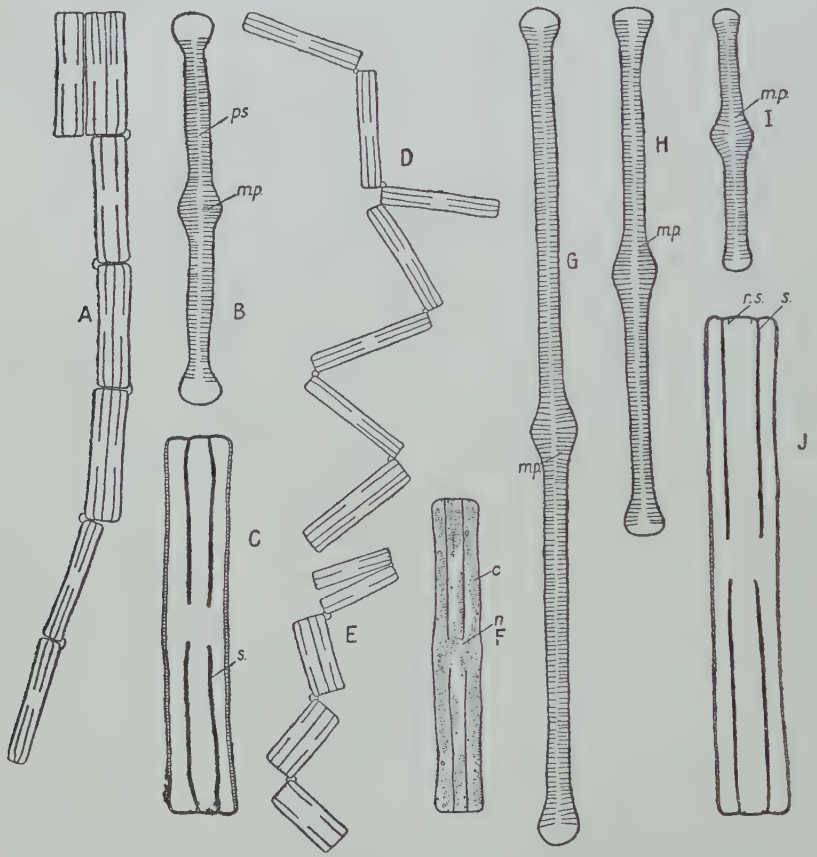


FIG. 1, A–C. *Tabellaria fenestrata* (Lyngb.) Kütz. A, colony morphology. B, valve. C, frustule in girdle view. B and C are drawn from type material.

D–J. *T. quadriseptata* nov. spec. D, E, colony morphology. F, living cell. G–I, valves. J, frustule in girdle view. *c*, chromatophore; *mp*, mucilage pore; *n*, nucleus; *ps*, pseudoraphe separating rows of transapical striae; *rs*, rudimentary septum; *s*, septum. (A, D, E, ×273; F, ×625; Rest, ×833.)

History). A slide labelled *T. fenestrata* by Smith himself (B.M. 24227) of material from Wareham contains frustules identical with those drawn in zigzag colonies in pl. 43 (the valve views, however, do not seem to have been drawn very accurately by Tuffen West). Similar specimens have been found in many tarns and large bog pools in the English Lake District, and also by the author in a pond near Wareham, Dorset, in October 1950. They consti-

tute a separate species whose chief diagnostic characters are the zigzag colony morphology, the 'definite' structure of the frustule, and a valve view in which the terminal inflations are approximately equal to the central inflation but are not capitate as in *T. fenestrata*. As will be seen in a later paper, the ecological distribution of this species is strikingly different from that of *T. fenestrata*. *T. quadriseptata* is found only in dystrophic or very oligotrophic waters, whereas *T. fenestrata* is characteristic of fairly eutrophic ponds and lakes and is never found in bog pools. *Tabellaria quadriseptata* nov. spec. is illustrated in Fig. 1, D–J, and diagnosed on p. 436.

Tabellaria flocculosa (Roth) Kützing

Although this species was drawn and described in 1703 by an anonymous naturalist (Philos. Trans. No. 288, p. 1499 and figs. 7 and 8), it was not named till 1797, when Roth described it as *Conferva flocculosa*. He mentions three longitudinal lines in each articulus (presumably a cell), although more than three are shown in some of his drawings, and, like Lyngbye, he was unable to recognize the septa as separate entities. He also describes the cells as hanging together at a point with a right angle between, thus forming a zigzag chain. I have been unable to obtain type material of *T. flocculosa*, but it may be concluded from the drawing and description that neither *T. fenestrata* nor *T. quadriseptata* were seen—not *T. fenestrata* because of the zigzag colony morphology, and not *T. quadriseptata* because of the numerous 'annuli' (septa) in the cells. As Roth's type material appears to have been lost, I am designating the example of Eulenstein's Diat. spec. typ. No. 50 (labelled spec. originale Lyngb. Tent. pag. 179, t. 61) in the British Museum (Natural History) (B.M. 22750) as neotype. Confirmation that this closely agrees with Roth's conception of the species is provided by a specimen of Mougeot and Nestler's Stirp. Crypt. Vogesorum No. 598 (*Diatoma flocculosum* De Cand., *Conferva flocculosa* Roth) annotated 'donum am Roth 1834' in a hand which is probably Shuttleworth's. Drawings made from this slide are reproduced in Fig. 2.

Tabellaria fenestrata var. *intermedia* Grunow in Van Heurck

This diatom was figured in Van Heurck's 'Synopsis des Diatomées de Belgique' (1880–5, pl. 52, figs. 6–8). There is no description, but the following remark, presumably by Grunow, accompanies the caption: 'This form and the small *T. flocculosa* var. *ambigua* Brügge closely connect the two species of the genus *Tabellaria*.' The original drawings show cells with 6 and 11 septa respectively, prominent rudimentary septa, and in valve view the apical inflations are not capitate. The slide of *T. fenestrata* in the B.M. set of Van Heurck's Types du Synopsis (No. 346, B.M. 26657), from which Fig. 3 has been made, contains specimens apparently identical with those drawn as var. *intermedia*, and in the absence of information to the contrary I take this to be the type material of var. *intermedia*. There are, on this slide, specimens agreeing with Grunow's and Van Heurck's concept of *T. flocculosa* and also specimens of the true *T. fenestrata* Lyngbye, as well as those figured as

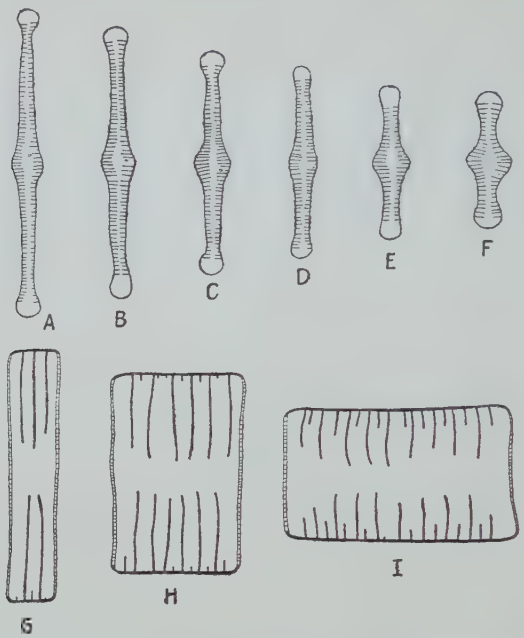


FIG. 2, A–I. *Tabellaria flocculosa* (Roth) Kütz. var. *flocculosa*. A–F, valves. G–I, frustules in girdle view. Drawn from the neotype (Eulenstein Diat. spec. typ. No. 50; B.M. 22750). (×833.)

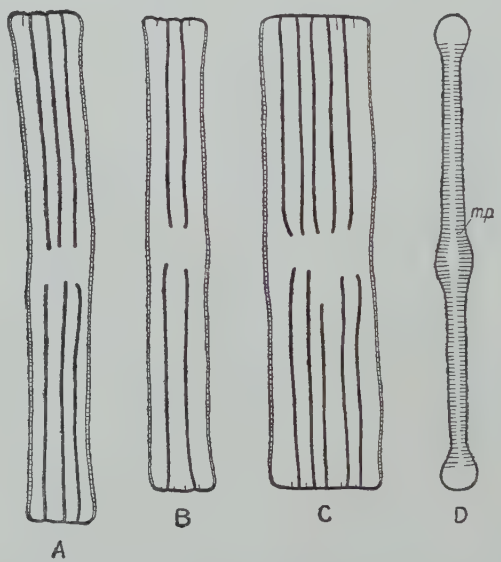


FIG. 3, A–D. *Tabellaria fenestrata* var. *intermedia* Grun. in V.H. A–C, frustules in girdle view. D, valve. Drawn from Van Heurck Types du Synopsis, No. 346 (= B.M. 26657). *mp*. mucilage pore. (×833.)

T. fenestrata var. *intermedia*. From measurements of these it is possible to see why Grunow regarded var. *intermedia* as bridging the gap between *T. fenestrata* and *T. flocculosa*. The length range of the *T. flocculosa* is 15–30 μ, that of *T. fenestrata* var. *intermedia* 53–87 μ, and of *T. fenestrata* 69–100 μ. The *T. fenestrata* in this sample therefore occurs only near the top of its length range, and owing to the similar length of the var. *intermedia* the two appear to intergrade. From evidence obtained in cultures it is not improbable that the *T. fenestrata* var. *intermedia* and *T. flocculosa* of Van Heurck's sample are members of a single morphological series. Their relationships will be discussed on p. 431.

T. fenestrata var. *asterionelloides* Grunow in Van Heurck

This variety was also drawn in Van Heurck's Synopsis (1880–5, pl. 52, fig. 9). There is no description in the text, and the only information about it is that the type locality is Hästefjord. The original drawing is very incomplete, but it may be seen that (1) the colony consists of six cells in a star, and (2) there is a slight tapering of the cell towards the outside of the colony. Owing to the apparent absence of a holotype I am using material from the type locality (Cleve and Möller, No. 75; B.M. 12815) as neotype. On this slide the length range of the diatom is 51–72 μ. About 70 per cent. of the colonies are star-shaped (Fig. 4, B), the rest being zigzag colonies in which the cells are at a similar angle to each other as in those of the first type. The valve may be seen in Fig. 4, C. The majority of the cells have 2, 3, or 4 septa, but two with 5 septa were observed.

It is widely held that *T. fenestrata* var. *asterionelloides* is merely a planktonic stellate form of *T. fenestrata* and that the two are interconvertible (e.g. Huber-Pestalozzi, 1942, p. 429, and Skuja, 1948, pp. 322–3). It may be noted, however, that *T. fenestrata* var. *asterionelloides* forms zigzags with an acute angle, whereas *T. fenestrata* forms 'straight-line' colonies. The valve view of *T. fenestrata* var. *asterionelloides* is totally different from *T. fenestrata* and the presence of occasional cells with five septa is another indication that *T. fenestrata* var. *asterionelloides* should not be referred to the species *T. fenestrata* (Lyngbye) Kützing.

2. *Experimental work on cultures*

Colony morphology. Unialgal clone cultures of *T. fenestrata* have been grown successfully (for particulars see Appendix II). No departure from the 'straight-line' type of colony morphology has taken place. Clone cultures of *T. flocculosa* have also been grown for periods up to 3 years and have shown no tendency towards a *fenestrata* type of colony morphology.

Cell morphology. No cells with more than four septa have been seen in cultures of *fenestrata* of valve length 57–41 μ (the length range observed up to the time of writing), i.e. all cells divide when four septa are visible. Twenty clones of *T. flocculosa* var. *flocculosa*, *T. fenestrata* var. *intermedia*, and *T.*

fenestrata var. *asterionelloides* isolated from various lakes have been investigated. In each of them the number of septa present at cell-division is variable at any given valve length. This seems to be a fundamental character and is the basis for uniting these taxa in the emended species *T. flocculosa* (Roth) Kützing. This revision is discussed in the next section.

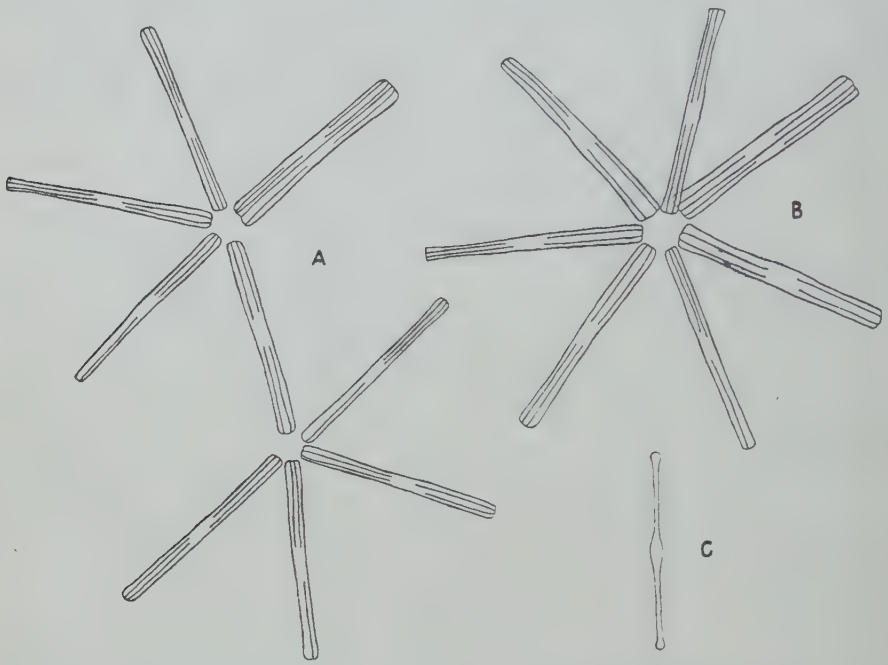


FIG. 4, A–C. *Tabellaria flocculosa* var. *asterionelloides* (Grun. in V.H.) nov. comb. A, B, colony morphology. C, valve contour. Drawn from the neotype (Cleve and Möller, No. 75; B.M. 12815). (×410.)

III. New Specific Limits of *T. flocculosa* (Roth) Kützing

Roth's original drawing of *T. flocculosa* shows a colony with frustules in girdle view. In the majority of these frustules the apical axis is 2–2½ times greater than the pervalvar axis, though in a few frustules they are approximately equal. These conditions are fulfilled in the neotype at valve length *c.* 32–36 μ. As the magnification of Roth's drawing is not stated, it is impossible to say whether this was so in the holotype. The neotype of *T. flocculosa* var. *flocculosa* shows that there is an increase in the mean length of the pervalvar axis with decrease in the length of the apical axis. In the longest cells (*c.* 44 μ) the pervalvar axis is only one-fifth or one-quarter the apical axis, whereas in the shortest cells (*c.* 19 μ) the pervalvar axis is almost twice the length of the apical axis. Is there any evidence for regarding this as a single morphological series? (It should be noted that the neotype of *T. flocculosa* is uncontaminated either by *T. fenestrata* or *T. quadriseptata*.) The problem has been investigated experimentally by isolating a single colony of an

epiphytic *Tabellaria* resembling Fig. 2, G and growing it in unialgal culture. The morphological range encountered during 3 years' sub-culturing of this clone culture ('Blelham 1') is shown in Fig. 5, B. It is obvious that the form changes very markedly with decrease in valve length in *T. flocculosa* var. *flocculosa*. The importance of this phenomenon was first recognized by Hassall (1845, i. 404–6) and his three drawings of *T. flocculosa* faithfully illustrate the diversity of form in a *flocculosa* series. Thus pl. 94, fig. 9 shows cells with apical axis half the length of the pervalvar axis and with 11–17 septa apiece; pl. 94, fig. 10 shows cells with apical axis twice the pervalvar axis and with 8–11 septa each, and pl. 96, fig. 11 depicts cells where the apical axis is six times the pervalvar axis and there are 2–6 septa per cell. Although Hassall did not understand the peculiarities of diatom growth, he drew attention to the increase in the number of septa accompanying decrease in valve length, and he was the first to include under *T. flocculosa* cells having only two septa and an apical axis greatly exceeding the pervalvar axis in length. His attempt to distinguish such cells from *T. fenestrata* was not wholly successful, and it seems likely that some of the specimens which he referred to *T. fenestrata* were in fact *T. quadriseptata*. The correctness of his judgement in identifying the specimens of pl. 96, fig. 11 as *T. flocculosa* has been proved by cultures. A colony of cells similar to that of Hassall's pl. 96, fig. 11 was isolated from Blelham Tarn, Lancashire, in October 1949. Fig. 5, A shows the changes which this clone ('Blelham 2') has undergone during 20 months' cultivation. It should be noted that the longest cells are dividing with only four septa.

In nature, even wider length variations are encountered. Some examples are shown in Fig. 5, C. A similar series was examined by Bachmann and the longer specimens erroneously called *T. fenestrata* merely because of their proportions in girdle view and the presence of four septa. This is the explanation for his view that '*T. fenestrata*' and '*T. flocculosa*' intergrade.

The smallest frustules in Blelham Culture 1 (see Fig. 5, B) do not differ from those described as *T. flocculosa* var. *compressa* Woodhead and Tweed (1948, p. 30). This variety was diagnosed on the squat compressed valve view, the valves being strongly tumid in the middle but not subcapitate at the poles. The breadth of the valves is given as 12–14 μ. Such specimens appear to be end members of a morphological series of *T. flocculosa* var. *flocculosa* and synonymous with it.

When we examine Van Heurck's Types du Synopsis No. 346 it is not difficult to believe that his '*T. flocculosa*' (valve length 15–30 μ) and '*T. fenestrata* var. *intermedia*' (valve length 53–87 μ) are members of a single morphological series. His '*T. fenestrata* var. *intermedia*' plus '*T. flocculosa*' differs from the neotype of *T. flocculosa* and the two cultures described above in having (1) a thicker frustule wall, and (2) a higher mean number of septa per cell. These characters appear separately in other populations and are not regarded as having taxonomic significance (see below). Moreover, populations resembling Van Heurck's *T. fenestrata* var. *intermedia* have not been found to have a different ecological or geographical distribution from

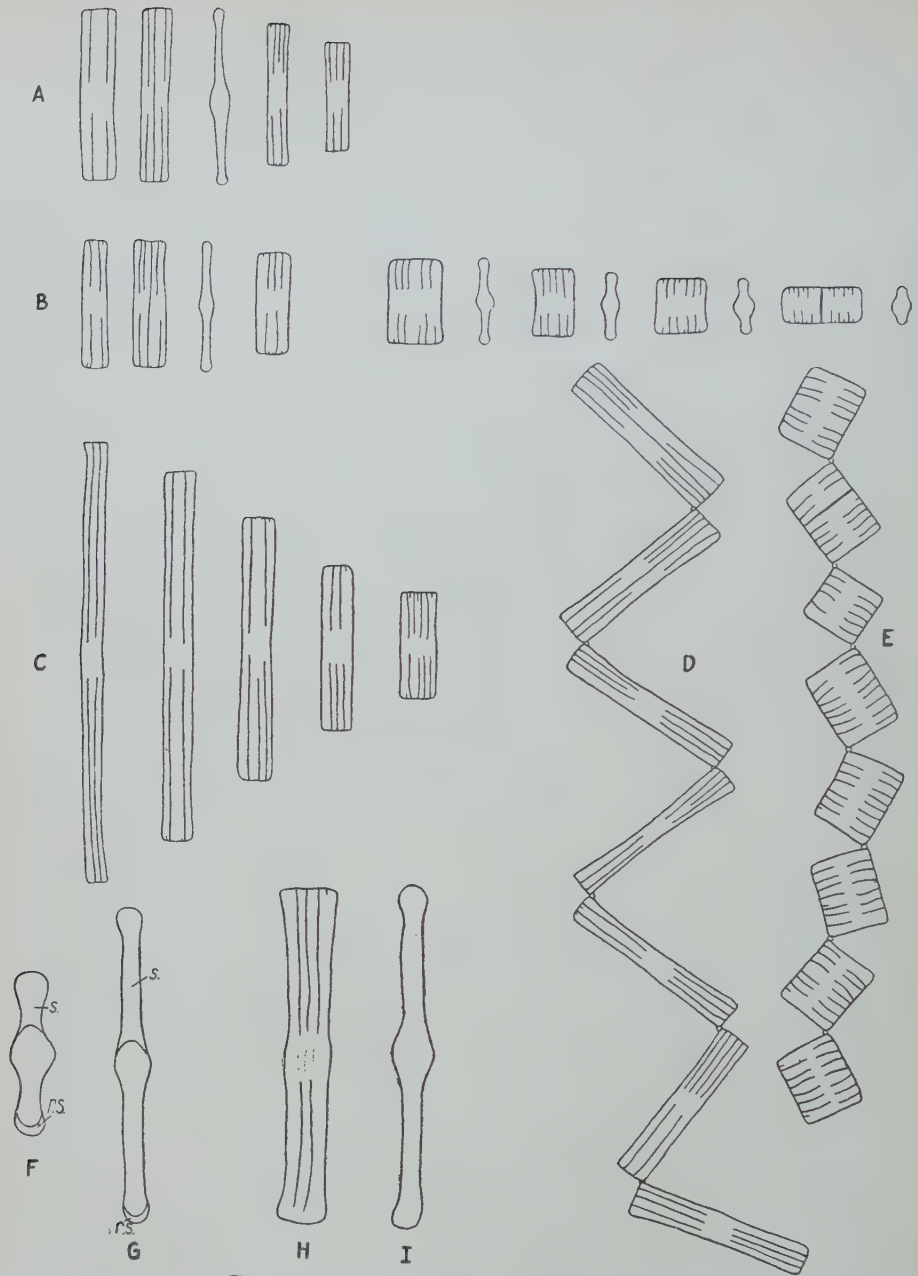


FIG. 5, A–I. *Tabellaria flocculosa* (Roth) Kütz.

A, range of structure shown in clone culture ('Blelham 2') of *T. flocculosa* (Roth) Kütz. var. *flocculosa*.

B, range of structure shown in clone culture ('Blelham 1') of *T. flocculosa* (Roth) Kütz. var. *flocculosa*.

C, range of structure shown in a single plankton sample from Derwentwater of *T. flocculosa* (Roth) Kütz. var. *flocculosa*.

D, E, colony morphology of epiphytic *T. flocculosa* (Roth) Kütz. var. *flocculosa* from Bassenthwaite.

F, G, *T. flocculosa* (Roth) Kütz. var. *flocculosa*. Isolated intercalary bands showing septa (*s.*) and rudimentary septa (*r.s.*). Epiphytic sample from Blelham Tarn.

H, I, *T. flocculosa* (Roth) Kütz. Frustules in girdle and valve view showing twist. Plankton sample from Ullswater. (A–E, ×410; F–I, ×937.)

T. flocculosa var. *flocculosa*, and the author therefore regards *T. fenestrata* var. *intermedia* as a synonym of *T. flocculosa* var. *flocculosa*.

The other variety which Van Heurck believed to link *T. fenestrata* with *T. flocculosa* was *T. flocculosa* var. *ambigua* Brügger. Brügger's type material was distributed as Wartmann and Schenk, Schweiz. Krypt. No. 339, and I have examined the specimen of this number in the British Museum (Natural History). It closely resembles the neotype of *T. flocculosa* and is therefore synonymous with it.

Reference has already been made to the variability of *T. flocculosa*. The characters by which frustules of the same valve length differ may be summarized as follows:

1. Frustule-wall thickness.
2. Degree of prominence of the central and apical inflations.
3. Curvature of septa.
4. Spacing of septa.
5. Maximum and minimum number of septa per cell.
6. Degree of development of the rudimentary septa.
7. Frustules twisted (Fig. 5, H, I) or not.
8. Angle between adjacent frustules.
9. Tendency to form star-shaped colonies.

Specimens with different combinations of these characters may be found in (*a*) a single sample, (*b*) different parts of the same lake, (*c*) samples from different lakes. It is only rarely that a homogeneous sample is encountered in nature. The combination of certain of these characters may produce such distinctive specimens that one might be tempted to give them varietal (or even specific) rank if one had not seen other populations in which the same characters were differently combined. Kützing's *T. ventricosa* (1844), distinguished by the prominent central inflation of the valve, and Grunow's *T. fenestrata* var. *intermedia* are both examples of this tendency.

We have already seen (p. 429) that *T. flocculosa* var. *flocculosa* and *T. fenestrata* var. *asterionelloides* are characterized by having an 'indefinite' frustule structure, i.e. the number of septa present at cell-division is variable. The type of frustule structure is regarded as fundamental to the classification of *Tabellaria*, distinguishing the species *T. quadriseptata* and *T. fenestrata* (with 'definite' frustules) from *T. flocculosa* and *T. binalis* (with 'indefinite' frustules). It has already been pointed out (p. 429) that in other characters, such as valve contour, the frequent presence of rudimentary septa, and in the mode of insertion of the septa, the affinities of *T. fenestrata* var. *asterionelloides* lie with *T. flocculosa* rather than with *T. fenestrata*.

Other taxa now to be considered are *T. fenestrata* var. *geniculata* A. Cleve and *T. flocculosa* var. *pelagica* Holmboe.

T. fenestrata var. *geniculata* A. Cleve was first described by A. Cleve (1899). The frustules have a geniculate flexion in the middle and a prominent central inflation to the valve. Cleve describes the colony morphology as parachute-like,

an effect produced by the geniculate flexion of the valves and the fact that separation of the frustules always takes place at the same pole. *T. fenestrata* var. *geniculata* is confined to the plankton of certain lakes in northern Scandinavia. The number of septa present at cell-division is indefinite, and because of this and the prominent central inflation of the valve, the variety should be referred to *T. flocculosa* and not *T. fenestrata*.

T. flocculosa var. *pelagica* Holmboe was discovered by Huitfeldt-Kaas and described first by Holmboe (1899). Holmboe described these planktonic colonies as star-shaped and he regarded *T. flocculosa* var. *pelagica* as analogous with *T. fenestrata* var. *asterionelloides*. Later (1906) Huitfeldt-Kaas added the following to Holmboe's description: 'the star-shaped colonies when fully developed form several, sometimes 4–5, spirally wound coils on account of which the colony, seen from the side, reminds one very much of a screw-thread'.

Teiling (1942, p. 68) gives the diagnostic characters of this variety as (1) the oblique twist of the frustules, and (2) the 'corkscrew' colony morphology, the frustules being so arranged that the outside edges are placed like the steps of a winding staircase, the twist being perpendicular to the axis of the screw. Teiling (1947, p. 230) describes the occurrence of this diatom in lakes 'characterized by a special oligotrophic phytoplankton association' and cites it, on account of its restricted geographical range, as an example of the unilocular origin of planktonic varieties.

We now have four taxa for inclusion within our conception of the species *T. flocculosa* (Roth) Kützing—those at present known as *T. flocculosa* var. *flocculosa*, *T. flocculosa* var. *pelagica*, *T. fenestrata* var. *asterionelloides*, and *T. fenestrata* var. *geniculata*. Ideally, varieties of one species should show similar degrees of discontinuity, but if the above taxa are retained this principle cannot be upheld. The most striking morphological peculiarity is shown by *T. fenestrata* var. *geniculata*, and this variety is further distinguished by a very restricted geographical range. The morphological isolation of *T. flocculosa* var. *pelagica* and *T. fenestrata* var. *asterionelloides* is not complete, for their diagnostic characters are found in different combinations in other populations. Unlike *T. fenestrata* var. *intermedia*, however, they show some degree of ecological or geographical or physiological discontinuity with *T. flocculosa* var. *flocculosa*.

The author regards *T. flocculosa* as a very variable species in which planktonic varieties have been, and are being, evolved. In some lakes *T. fenestrata* var. *asterionelloides* is a perennial in the plankton, showing complete morphological and physiological isolation from *T. flocculosa* var. *flocculosa*; in other lakes stellate plankton colonies are merely ephemeral environmental forms of *T. flocculosa* var. *flocculosa*. To the taxonomist with a morphological outlook these are untidy categories, but they are in frequent use by limnologists and should not be jettisoned before their relationships have been more adequately examined. Ascription of varietal limits to these taxa is postponed until further morphological and ecological data have been examined; the descrip-

tions of the four varieties of *T. flocculosa* given in the conspectus are not meant to be exhaustive. *T. fenestrata* var. *Willei* Huitfeldt-Kaas and *T. fenestrata* var. *lacustris* Meister will also be considered in a later paper on planktonic varieties of *Tabellaria*.

IV. New Specific Limits of *T. fenestrata* (Lyngbye) Kützing

Reasons have already been given for excluding from this species *T. fenestrata* var. *intermedia*, *T. fenestrata* var. *geniculata*, and *T. fenestrata* var. *asterionelloides*, the only varieties separate from the type which are nowadays recognized as distinct.

The length range of the type material is 49–73 μ. Longer and shorter specimens than this are frequently encountered, and some are illustrated in Fig. 6, A–D. The total length range observed by the author is 25–116 μ. Below about 33 μ frustules with five septa are occasionally found (Fig. 6, E, F), and it would appear that at the extreme lower limit of the length range the silicification of septa gets out of phase with the silicification of new valves. Frustules below 33 μ are extremely rare. It is very possible that the upper limit of the length range will be extended as more material is examined. The measurements of other authors have not been quoted owing to the uncertainty of their identifications of *T. fenestrata*.

During this investigation no variations have been encountered in *T. fenestrata* from the same or different lakes. There is thus a great contrast between this species and *T. flocculosa* in their degree of morphological plasticity, suggesting that the genetic basis of the two species is very different.

V. Practical Difficulties in Identification

Tabellaria fenestrata is always identifiable by its colony morphology: the 'straight-line' type is found even in colonies of very short cells (25 μ valve length). This, together with the bent insertion of the septa and the absence of rudimentary septa, is the most reliable character, since the apical inflations are not distinctly capitate in short valves, and anomalous cells with more than four septa are occasionally produced in cells below 33 μ valve-length (Fig. 6, E, F).

For the greater part of its length range *T. flocculosa* is readily distinguishable from *T. quadriseptata* by the presence of multiseptate cells. Difficulties are liable to arise with (1) *T. flocculosa* cells having four or fewer septa; (2) *T. quadriseptata* cells at the extreme lower end of their length range, when (as in *T. fenestrata*) anomalous cells with more than four septa are produced.

In doubtful instances of the first kind, reference should always be made to other cells in the same colony. Preparations made by incineration or with hydrogen peroxide (see Appendix III) are best for this purpose, since most of the colonies remain intact. If the species in question is *T. flocculosa* it is very probable that other cells in the colony will be found to have more than four septa.

The wide spacing of the central septa (those adjacent to the girdle) is characteristic of mature cells of *T. quadriseptata* (and *T. fenestrata*). *T. quadriseptata* cells at the lower limit of their length range are shown in Fig. 6, M, N. They may be compared with *T. flocculosa* of similar valve length (Fig. 6, O).

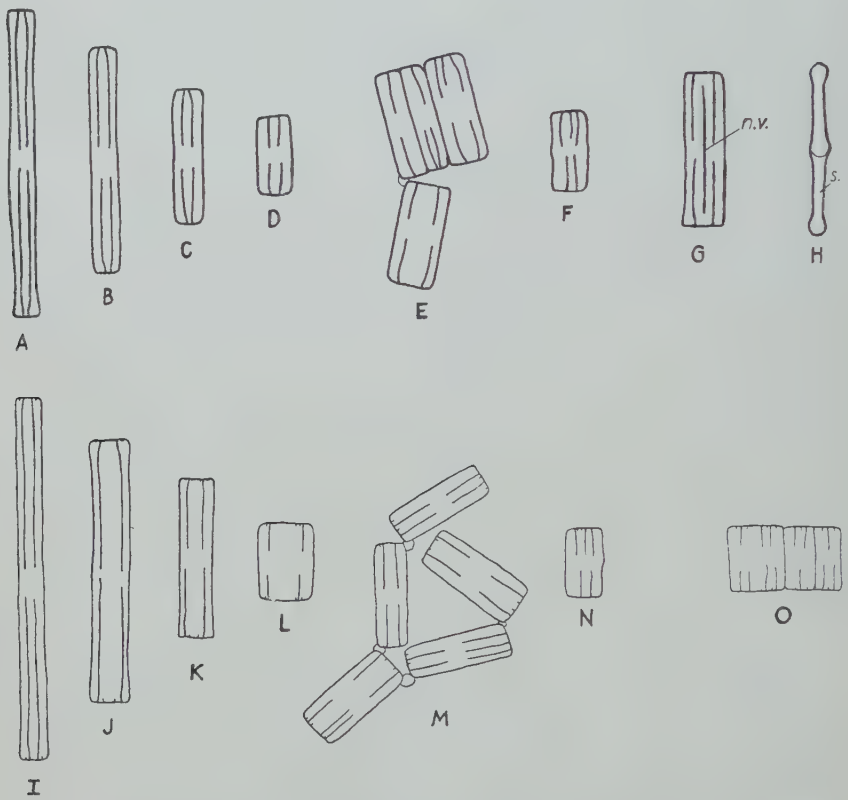


FIG. 6, A–H. *Tabellaria fenestrata* (Lyngb.) Kütz. A–D, frustules in girdle view. E, F, abnormal specimens. G, dividing cell showing new valves (*n.v.*). H, intercalary band and septum (*s.*).

I–N. *T. quadriseptata* nov. spec. I–L, frustules in girdle view. M, N, abnormal specimens.

O, *T. flocculosa* for comparison with N. (All, ×410.)

Identification of these species by valve view alone is not always possible. Separation of *T. fenestrata* from *T. quadriseptata* may be made on valve contour (in *T. fenestrata* the terminal inflations are distinctly capitate, whereas in *T. quadriseptata* the terminal inflation gradually tapers into the shaft) and by the fact that in *T. fenestrata* the mucilage pore on the central inflation is situated towards the centre of that structure, whereas in *T. quadriseptata* it is near one end. Sometimes, e.g. when the central inflation is very prominent, *T. flocculosa* may be distinguished with ease from the other two species, but

T. flocculosa is a very variable species showing many different types of valve contour, some of which approximate to that of *T. quadriseptata*. Moreover, the mucilage pore is variable in position in *T. flocculosa* and it has not been possible to find any significant differences in striae density between the three species. Over the same valve length range (50–100 μ) all three species show similar numbers of striae (*T. fenestrata* 15–18 per 10 μ; *T. flocculosa* 14–17 per 10 μ; *T. quadriseptata* 14–18 per 10 μ).

The following table summarizes the diagnostic characters of these three species, the specific limits being defined in the conspectus below. Atypical specimens at the lower limit of their length range are excluded for the sake of simplicity.

	T. fenestrata	*T. quadriseptata*	*T. flocculosa*
Colony morphology	Straight-line	Zigzag	Zigzag or star or corkscrew
Number of septa at cell-division	4	4	4–32 or more
Rudimentary septa	Absent	Present or absent	Present or absent
Length range	25–116 μ	23–129 μ	6–130 μ
Valve contour	Three inflations approximately equal in width: apical inflations distinctly capitate	Three inflations approximately equal in width: apical inflations tapering gradually to shaft	Very variable; central inflation often wider than apical ones: apical inflations not distinctly capitate
Position of mucilage pore on central inflation	Towards centre of inflation	Near one end of inflation	Very variable
Habitat	In fairly eutrophic ponds and lakes, not bog pools	In dystrophic and very oligotrophic waters	Widely distributed, often found with the two previous species

It is worth noting that the minimum length of *T. flocculosa* is much lower than that of *T. quadriseptata* and *T. fenestrata*.

VI. Conspectus of the Genus

Full synonymies are not given in this conspectus since they are available in Hustedt (1931, pp. 25–31), and those given below are intended to indicate where my views on the relationships of the various taxa differ from his.

Tabellaria fenestrata (Lyngbye) Kützing, Die Kies. Bacill., p. 127 (1844).
Diatoma fenestratum Lyngbye, Tent. Hydrophyt. Dan., p. 180 (1819).
excl. *T. fenestrata* var. *intermedia* Grunow in Van Heurck.
T. fenestrata var. *asterionelloides* Grunow in Van Heurck.
T. fenestrata var. *geniculata* A. Cleve.

Colonies mainly composed of cells joined at an angle of 180° and therefore lying in straight lines; cells 25–116 μ long, with 2, 3, or 4 septa. Rudimentary

septa absent. Septum with a sharp bend very close to its point of insertion so that this is farther from the valve than its free end. Valve view with three approximately equal inflations; apical inflations distinctly capitate. Striae 15–18 in 10 μ. *Habitat*: attached to substrata in fresh waters; very rarely in plankton, and then never in large quantities.

Tabellaria quadriseptata nov. spec.

Coloniae 'zigzag' ad instar. Frustula 2, 3, aut 4 septis instructa. Valvae 23–129 μ longae, inflationibus fere aequalibus. Inflationes terminales in partem valvae directam leniter fastigantes. Porus mucilaginis inconspicuus, prope fine inflationis centralis positus. Striae 14–18 in 10 μ. *Habitat*: ad substrata in aquis dystrophis et valde oligotrophis adhaerens.

Typus in Herb. Mus. Brit., coll. Diat., No. B.M. 36263, ab 'Tarn-at-Leaves', Cumberland, Anglia; paratypi Nos. B.M. 36264, 36265, ab eodem loco.

Zigzag colonies, frustules with 2, 3, or 4 septa. Valves 23–129 μ long with three approximately equal inflations. Terminal inflations gradually tapering towards the shaft. Mucilage pore inconspicuous, situated at periphery of central inflation. Striae 14–18 in 10 μ. *Habitat*: attached to substrata in dystrophic and very oligotrophic waters.

Type material from Tarn-at-Leaves, Cumberland, England, May 13, 1951. Holotype in British Museum (Natural History), Herb. Diat., No. 36263; paratypes Nos. 36264 and 36265.

Tabellaria flocculosa (Roth) Kützing, Die Kies. Bacill., p. 127 (1844).
Conferva flocculosa Roth, Cat. Bot. i, p. 192 (1797).
Bacillaria flocculosa (Roth) Leiblein in Flora, x, p. 288 (1827).[1]

Colonies zigzag, corkscrew-, parachute-, or star-shaped; frustules symmetrical or asymmetrical in the valvar and apical planes; septa 2–32 or more per cell; rudimentary septa often present. Valves 6–130 μ, with central inflation as broad as, or broader than, the apical inflations, which are not distinctly capitate. *Habitat*: fresh waters, either attached or planktonic.

var. *flocculosa* (Roth) nov. comb.
Conferva flocculosa Roth, loc. cit.
Tabellaria flocculosa (Roth) Kützing, loc. cit.
Tabellaria flocculosa var. *ambigua* Brügger in Jber. naturf. Ges. Graubünden, N.F., viii, p. 289 (1863).
Tabellaria fenestrata var. *intermedia* Grunow in Van Heurck, Syn. Diat. Belg., pl. 52, figs. 6–8 (1881).
Tabellaria flocculosa var. *compressa* Woodhead and Tweed in Northw. Nat., xxiii, Nos. 1–4, p. 30 (1948).

Colonies usually zigzag, occasionally star-shaped. Colonies usually attached, sometimes planktonic.

This new combination is made in accordance with Art. 28 *bis* (Lanjouw,

[1] Hustedt (loc. cit.) cites *Bacillaria tabellaris* Ehr. as a synonym of *T. flocculosa*, but some of Ehrenberg's figures appear to represent *Fragilaria* sp.

1950, p. 68) of the International Rules, which was incorporated in them at Stockholm, 1950.

var. *pelagica* Holmboe in Arch. Math. Naturv. xxii (1), p. 27 (1900). emend. Teiling in Bot. Notiser, 1942, p. 68 (1942).

Colonies corkscrew-shaped, planktonic. Frustules with oblique twist.

var. *asterionelloides* (Grunow in Van Heurck) nov. comb.

Tabellaria fenestrata var. *asterionelloides* Grunow in Van Heurck, Syn. Diat. Belg. pl. 52, fig. 9 (1881).

Colonies usually star-shaped, occasionally zigzag. Planktonic.

var. *geniculata* (A. Cleve) nov. comb.

Tabellaria fenestrata var. *geniculata* A. Cleve in Öfvers. VetenskAkad. Förh. Stockh. lvi, p. 831 (1899).

Colonies parachute-shaped, planktonic. Frustules geniculate in valve view with prominent central inflation.

Tabellaria binalis (Ehrenberg) Grunow in Van Heurck, Syn. Diat. Belg. pl. 44, fig. 23 (1881).

Fragilaria? *binalis* Ehrenberg, Mikrogeologie, pl. 14, fig. 52 (1854).

Colonies band-shaped, occasionally showing transitions to the zigzag condition. Dissection of the colony in this way takes place at rather rare intervals along the band and the separation of adjacent cells does not seem to be accompanied by the formation of a mucilage pad as in the other three species. Frustules rectangular in girdle view, with pervalvar axis greater than or equal to the apical axis. Valves constricted in the centre, 8–22 μ long. Septa short and numerous and not usually at right angles to the pervalvar axis. The number of septa present at cell-division is indefinite. *Habitat*: the littoral regions of very oligotrophic lakes, where it occurs in the matrix formed by other algae, aquatic mosses, &c.

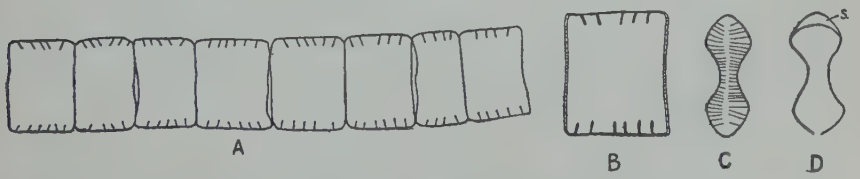


FIG. 7, A–D. *Tabellaria binalis* (Ehr.) Grun. in V.H.
A, colony morphology. B, frustule in girdle view. C, valve. D, intercalary band and septum (*s.*). (A, ×625; B–D, ×833.)

This species was discovered by the author in two Cumberland tarns (Scoat Tarn, near Wastwater, and High Nook Tarn, near Loweswater) in the summer of 1950 (first British records). The material so far obtained from these localities is of valve length 13–22 μ, and the lower valve length quoted above is taken from Hustedt (1931, pp. 30–31).

Acknowledgements

My thanks are due to the Director and Staff of the Freshwater Biological Association, and especially to Dr. J. W. G. Lund, who first suggested these problems to me. I gratefully acknowledge the valuable assistance given me throughout this investigation by Mr. R. Ross, for affording me every facility for consulting the relevant diatom collections and literature at the British Museum (Natural History), and for his detailed criticism of the manuscript. I am indebted to Mr. and Mrs. R. Ross for the Latin diagnosis, to Dr. J. Boye Petersen for kindly sending me type material of *T. fenestrata*, and to Mr. F. C. Wise for bringing to my notice the earliest known reference to *Tabellaria*.

Summary

1. Existing accounts of *Tabellaria* are untrustworthy because *T. fenestrata* (Lyngb.) Kütz. has been confused with *T. quadriseptata* nov. spec. as well as with long specimens of *T. flocculosa* (Roth) Kütz. var. *flocculosa*.

2. Type material of *T. fenestrata* (Lyngb.) Kütz. has been examined and other specimens of a wide range of length, and the species is redescribed from these and from unialgal clone cultures. Attention is drawn to the distinctive 'straight-line' colony morphology of *T. fenestrata*, illustrated by Lyngbye but neglected by the vast majority of later authors. Living material throughout the known length range, and cultures, show this to be a character of diagnostic importance.

3. A neotype of *T. flocculosa* (Roth) Kütz. var. *flocculosa* is drawn and described and the specific limits of *T. flocculosa* are discussed. The present classification of *Tabellaria* is shown to be unnatural and is revised on the basis of growth differences which are regarded as fundamental. In the 'definite' group (*T. fenestrata* and *T. quadriseptata*) cell-division always occurs when the mother-cell contains four septa; in the 'indefinite' group (*T. flocculosa*, *T. fenestrata* vars. *asterionelloides*, *geniculata*, and *intermedia*) there are four or more septa, the number varying at any given valve length.

4. *T. fenestrata* var. *intermedia* Grun. in V.H. is shown to be a synonym of *T. flocculosa* var. *flocculosa*. *T. fenestrata* var. *asterionelloides* Grun. in V.H. and *T. fenestrata* var. *geniculata* A. Cleve are transferred to the species *T. flocculosa*.

5. The specific limits of *T. fenestrata* are discussed. *T. fenestrata* and *T. flocculosa* are shown to be very different in their degree of morphological plasticity. Bachmann's view that these two species intergrade is shown to be erroneous.

6. A diagnosis of *T. quadriseptata* nov. spec. is given and a table summarizes the chief characters of this species, *T. fenestrata*, and *T. flocculosa*.

7. The only other known species of *Tabellaria*, *T. binalis* (Ehr.) Grun. in V.H., has been found by the author in two Cumberland tarns. These are the first British records. The paper concludes with a conspectus of the genus.

Appendix I

The colony morphology of *T. fenestrata* is correctly portrayed in the following works: Kützing, F. T. (1865), Die kieselschaligen Bacillarien, pl. 18, fig. 2; Ralfs, J. (1843), Ann. Mag. nat. Hist., vol. xi, Series I, pl. 9, fig. 4.

Various diatom collections purporting to contain *T. fenestrata* have been examined. Some are incorrectly named, whilst others, although containing some *T. fenestrata*, consist predominantly of long specimens of *T. flocculosa*. The best gatherings of *T. fenestrata* are: Desmazières, Plantes cryptogames de France, Series 2, No. 511; Jack, Leiner, and Stizenberger, Kryptogamen Badens, No. 350; Rabenhorst, Algen Europas, No. 1361.

The following are mixtures of *T. fenestrata* and long specimens of *T. flocculosa*: Tempère and Peragallo, Edition 2, Nos. 212 and 249.

No *T. fenestrata* has been detected in the following collections, which consist entirely of *T. flocculosa*: H. L. Smith, No. 588 (= B.M. 26149); Tempère and Peragallo, Edition 2, No. 82.

Appendix II

The culture fluid consists of (1) Chu's solution 10 (1942, p. 298) made up with filtered Windermere lake-water instead of distilled water and with added mud-extract (see Lund, 1949, p. 393); (2) a solution of ferric citrate and citric acid prepared and used as in Rodhe's solution 8 (Rodhe, 1948, p. 57).

Appendix III

Methods of preparing Material

(*a*) *Incineration*. A drop of the diatomaceous sample is placed on a coverslip and is heated strongly for a few minutes on a thin brass bar. The coverslip may then be mounted directly in some permanent material such as Stafford Allen's Sirax. With this method the form of a *Tabellaria* colony is maintained, and since the frustules come to lie on their girdle faces, the septa per cell may be counted with ease.

(*b*) *Hydrogen peroxide*. A drop of the diatomaceous sample is placed on a slide and treated with successive drops of 'Analar' 100-volume hydrogen peroxide. The reaction may be accelerated by gentle warming on a hot-plate. When the preparation is thoroughly dry it may be mounted in the manner suggested above. The degree of disintegration of the colonies and frustules depends on the quantity of peroxide used and the heat applied. With prolonged application of hydrogen peroxide many of the frustules fall into their constituent parts.

Literature Cited

Bachmann, H., 1907: Vergleichende Studien über das Phytoplankton. Arch. Hydrobiol. Plankt., Bd. iii, pp. 1–91.

Chu, S. P., 1942: The Influence of the Mineral Composition of the Medium on the Growth of Planktonic Algae. I. Methods and Culture Media. J. Ecol., xxx, No. 2, pp. 284–325.

Cleve, A., 1899: Notes on the Plankton of Some Lakes in Lule Lappmark, Sweden. Öfvers. VetenskAkad. Förh., Stockh., lvi. 825–35.

Hassall, A. H., 1845: History of the British Freshwater Algae. Vol. i, London.

Holmboe, J., 1899: Undersøgelser over Norske Ferskvandsdiatoméer. I. Diatoméer fra indsjøer i det sydlige Norge. Arch. Math. Naturvid., xxii (1), pp. 1–72.
Huber-Pestalozzi, G., 1942: Das Phytoplankton des Süsswassers. Die Binnengewässer, Teil 2, 2 Hälfte, Stuttgart.
Huitfeldt-Kaas, H., 1906: Planktonundersøgelser i norske vande. Christiania.
Hustedt, F., 1931: Bacillariophyta. Rabenhorst, Kryptogamen-Fl. 7, Teil 2. Leipzig.
Kützing, F. T., 1844: Die kieselschaligen Bacillarien, Nordhausen. 2nd edition, Nordhausen, 1865.
Lund, J. W. G., 1949: Studies on Asterionella. I. The Origin and Nature of Cells producing Seasonal Maxima. J. Ecol., xxxvii. 389–419.
Lyngbye, H. C., 1819: Hydrophytologiae Danicae. Hafniae.
Rodhe, W., 1948: Environmental Requirements of Freshwater Plankton Algae. Symb. bot. Upsaliens, x (1), pp. 1–149.
Roth, A. W., 1797: Catalecta botanica. Lipsiae.
Skuja, H., 1948: Taxonomie des Phytoplanktons einiger Seen in Uppland, Schweden. Symb. bot. Upsaliens, ix (3), pp. 1–399.
Smith, W., 1856: Synopsis of the British Diatomaceae. London.
Teiling, E., 1942: Schwedische Planktonalgen 3. Bot. Notiser, 1942, pp. 63–68.
—— 1947: Staurastrum planktonicum and St. pingue. A Study of Planktic Evolution. Svensk bot. Tidskr., xli. 218–234.
Van Heurck, H., 1880–5: Synopsis des Diatomées de Belgique. Antwerp.
Woodhead, N., and Tweed, R. D., 1948: Some new Welsh Algae. Northw. Nat., xxiii, Nos. 1–4, pp. 22–32.

A Physiological Study of Embryo Development in *Heracleum sphondylium* L.

I. The Effect of Temperature on Embryo Development[1]

BY

PEARL STOKES

(*University College, London*)

With three Figures in the Text

Abstract

Growth of embryos within the seed of *Heracleum sphondylium* proceeds more rapidly at low temperature than at room temperature. At 2° growth is exponential and in 9 weeks the size is increased 4 times and the dry weight 25 times. At 15° there is a progressive falling off in the rate of growth, and growth ceases when both size and dry weight have been only doubled.

Introduction

THE dormant seeds of *Heracleum sphondylium* contain only very rudimentary embryos, the bulk of the seed consisting of endosperm. Before germination the greater part of the endosperm reserves are absorbed by the embryo, which develops from a heart-shaped ball of cells comprising only 0·4 per cent. of the seed dry weight to a seedling weighing 30 per cent. of the seed dry weight. At germination the cells of the radicle elongate, causing a rapid extension growth, and the cotyledons continue to absorb the remainder of the endosperm before the seedling becomes independent.

The development of the embryo within the seed is affected by temperature, for germination occurs only after a period of 2 to 3 months at low temperature. This low-temperature treatment is known as 'after-ripening' and is a familiar requirement for the germination of many seeds. The case of *Heracleum sphondylium* is rather exceptional, however, for as is shown in this paper, after-ripening is concerned with the growth of the embryo, while in most other species known the embryo is already fully grown (Crocker, 1948; Crocker and Barton, 1948; Davis and Rose, 1912; Haut, 1933; Roberts, 1924; Steinbauer, 1937).

Material and Method

Ripe fruits of *Heracleum sphondylium* L. were collected in the vicinity of Bristol and north London. After harvesting, fruits were laid out to

[1] Part of this work was incorporated in a thesis presented for the degree of Doctor of Philosophy at the University of Bristol, 1950.

[Annals of Botany, N.S. Vol. XVI, No. 63, July, 1952.]

dry at 23° C. before being stored in ground-glass stoppered jars at room temperature.

The fruits were soaked overnight. The seeds were then dissected under a binocular dissecting microscope using very small forceps and a needle-knife. All those seeds which appeared healthy and in good condition were used, so that no active principle of choice was operative in the selection of samples.

The effects of room temperature and low temperature on embryo development are compared. For this purpose, a refrigerator with a temperature

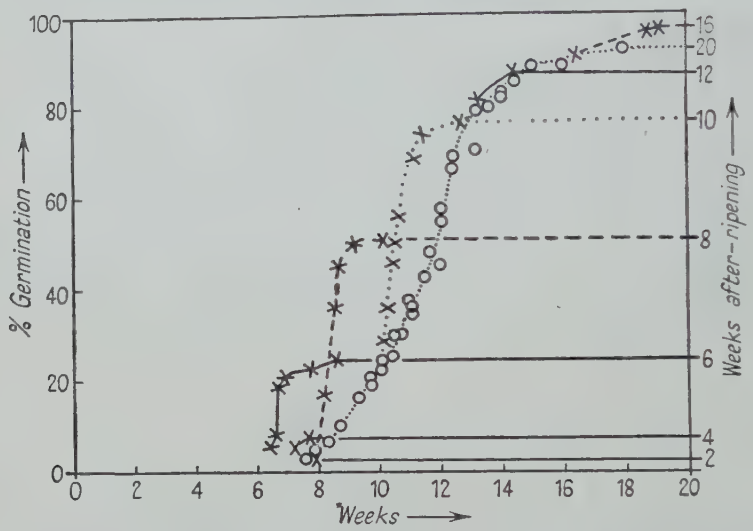


FIG. 1. Percentage germination of seeds given 2, 4, 6, 8, 10, 12, 16, and 20 weeks after-ripening at 2° C. before being transferred to room temperature. x denotes germinations at room temperature. o denotes germinations at low temperature.

fluctuating between 2° C. and 5° C. was used for comparison with a constant-temperature room maintained at 15° C.

I. Temperature Requirements for Germination

In order to study the effect of temperature on germination, seeds, excised from the pericarps, were set up on strips of filter-paper on glass slides 3 in. × ¾ in. These stood in 5 ml. of distilled water in 6 in. × 1 in. boiling tubes plugged with cotton-wool. The filter-paper adhered to the slide and was kept uniformly moist. In this way seeds placed on the slope were kept constantly damp and the dangers of drying out and flooding, both of which are difficult to control in flat dishes, were avoided.

The seeds were put up in sets of 40 for each treatment, 4 tubes with 10 seeds in each. Different sets were given 2, 4, 6, 8, 10, 12, 16, and 20 weeks in a refrigerator before being transferred to a room temperature of 15° C. The number of germinations was noted periodically and the results for each set expressed as a percentage. Thus at any one date the number given is the total percentage of germinations which have taken place up to that date.

Results. Germination was found to take place both in the refrigerator and after removal to the warmth, but results given in Fig. 1 show that a minimum of 6 weeks at low temperature is necessary to obtain a 25 per cent. germination. A full germination is only obtained after 3 months' exposure to cold.

It is apparent, however, that the necessity for low temperature ends some time before germination in the refrigerator. Thus 50 per cent. of the seeds will germinate immediately in the warmth after 8 weeks after-ripening, but a 50 per cent. germination is not obtained until after 12 weeks when seeds are left in the refrigerator. This delay in germination at low temperature indicates that after the completion of after-ripening the actual emergence of the radicle is a normal growth process which is subject to temperature in the normal way, having a $Q10$ of between 2 and 3.

II. The Effect of Temperature on the Embryo Growth and the Transfer of Nutrients from the Endosperm

It was not possible to follow the growth of individual embryos over a period of time since the excision necessary for examination brings growth to an end *ipso facto*. Large numbers of seeds were therefore examined at successive stages during development and conclusions based on an 'average' behaviour. Sufficient numbers of seeds to enable periodic sampling were put to develop concurrently in a refrigerator and at room temperature for comparison.

After being washed seeds were placed flat side down on moist filter-paper. Flat dishes covered with glass plates were used for setting up large numbers of seeds for dissection. Half of these were stored at 2° C. and the other half at 15° C.

Increase of embryo size within the seed is accompanied by morphological development and there is a close correlation between length and stage development. Embryo length is therefore used as a suitable criterion for expressing morphological development on a quantitative basis. Length measurements were made on samples of 40 embryos from each temperature at half-weekly intervals, 1-weekly intervals, and 3-weekly intervals on the harvests of 1950, 1948, and 1947 respectively.

At intervals of 3 weeks, samples were removed from both temperatures for dry-weight determinations of both whole seeds and embryos. Material for each weighing was composed of 250 seeds or embryos.

Embryo growth. Length measurements are recorded for seed of 1947, 1948, and 1950. The initial mean sizes differ each year but the growth curves are very similar. Fig. 2 shows the combined data for all three harvests plotted as multiples of the original mean size.

From Fig. 2 it will be seen that very much more development takes place at 2° C. than at 15° C. Thus during 9 weeks at low temperature embryos develop to 4 times their original size, while at room temperature they only double their original size.

From Fig. 1 it will be seen that 50 per cent. of the seeds have completed

their after-ripening after 8 weeks at low temperature. The 'average' seed, with which the measurements in this section are concerned, are therefore described as 'after-ripened' after 8 weeks at 2° C. and germinated after 12 weeks at 2° C.

The implication of the effect of temperature on growth is, therefore, that while germinating size is reached at 2° C., only half that size is attained at 15°. Examination of the curves shows that at the actual commencement of

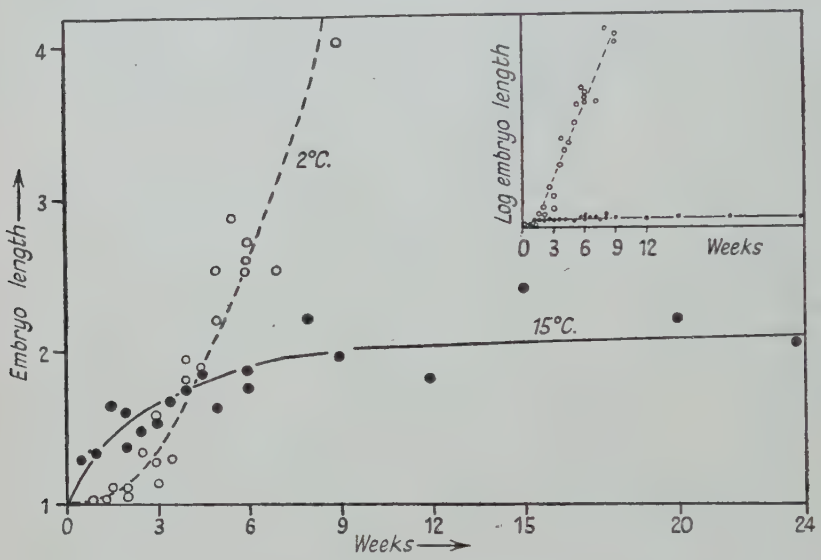


FIG. 2. Embryo lengths plotted as multiples of the original mean size.

growth the initial rate is considerably higher at room temperature than at 2°, as would be normally expected, but at 15° the rate of growth shows a progressive falling off, so that in spite of the rapid increase during the first week there is virtually no further development after 6 weeks. This, therefore, is the reason for the failure to germinate at room temperature without after-ripening. Growth of the embryo is completely arrested for some reason before it has reached more than half the size necessary for germination.

The form of the growth curve at 15° suggests that growth at this temperature is being retarded by some limiting factor of progressively increasing severity. The exponential curve of growth at 2°, on the other hand, indicates that there is no such restriction operative at low temperature.

It may be mentioned here that there are two possible alternative causes of the cessation of growth. Firstly there is the development of a condition, such as CO_2 narcosis, which would inhibit the growth process. Secondly, it must be remembered that the growth of the embryo is dependent on a supply of nutrients from the endosperm. If these are not forthcoming at room temperature, growth would be unable to continue.

Transfer of nutrients from the endosperm to the embryo. Determinations of dry weight were made in 1947 and 1948. In both harvests the embryo comprised only 0·3 to 0·4 per cent. of the total seed dry weight and behaviour in both years was essentially the same.

From Fig. 3 it will be seen that the rate of transference of materials from the endosperm to the embryo is also much higher at 2° C. than at 15° C. Thus, after 9 weeks at low temperature the embryo weight is 25 times the

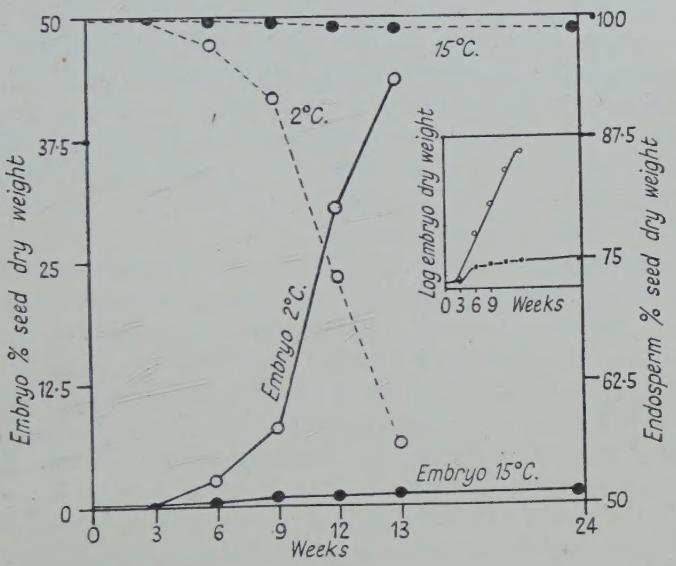


FIG. 3. Dry weights of embryos and endosperms expressed as a percentage of seed dry weight.

initial dry weight, while at room temperature the weight is just doubled during this time. Thus becomes apparent the important fact that whereas the difference in growth in size between embryos growing at 2° and 15° was ×2 after 9 weeks, the difference in weight is about ×12. Embryos at 15° therefore, which must literally be starved, grow nevertheless to a length of half that reached by comparable embryos at 2°, an observation which suggests that no substance inhibiting growth itself is present. The only alternative explanation of the cessation of growth at 15°, therefore, appears to be that the nutrients stored in the endosperm are simply not available at this temperature.

It is interesting to note that during the first 3 weeks of growth the increase in dry weight is small and of approximately the same magnitude at both temperatures. It is only after the third week that the discrepancy occurs. At room temperature the rate of growth then shows a rapid falling off, while at low temperature the uptake of nutrients becomes so rapid that surplus sugars are available for the formation of dextrins and starch in the embryo (Stokes, 1950).

Discussion and Conclusion

Before germination can take place there are two processes which must be completed. The first is morphological development, and the second is an accumulation of soluble foods without which the sudden expansion growth at germination cannot take place.

There is very little reserve material in the embryo of *Heracleum* itself. The embryo is, therefore, largely dependent upon the transference of nutrients from the endosperm both for growth and accumulation. Transference only takes place at low temperature, so that neither of the conditions for germination can be fulfilled without low-temperature treatment. As has been pointed out, this might be due to an inability of the embryo to take up nutrients and grow at room temperature, which could be attributed to the development of CO_2 narcosis of the embryo, or some similar inhibiting condition. Alternatively the endosperm could be present in a form only made available for embryo metabolism at low temperature.

In this connexion it is significant that the rate of embryo growth shows a normal response to temperature, having a $Q10$ of between 2 and 3, at the beginning of development, and again after the attainment of a nutritional status which occurs when the dry weight is 25 times the original.

During the first 10 days there are apparently sufficient nutrients available in and around the embryo at both normal and low temperatures. The reason that it is only later, after 3 weeks, that low temperature increases the relative growth rate is probably because it is only when the nutrients originally available to the embryo are exhausted that growth is limited by the rate of uptake of endosperm food reserves. Since, as has been shown, this rate of uptake is greater at low temperature, there is consequently a higher rate of growth at low temperature which continues only until sufficient materials are accumulated in the embryo for the rate of uptake to be no longer limiting.

It is suggested, therefore, that starvation is limiting growth at room temperature, and the action of low temperature in promoting growth is to bring about the mobilization of the food in the endosperm.

Summary

1. Germination of seeds of *Heracleum sphondylium* will only take place after a period of 2 to 3 months at low temperature.

2. Embryo growth is exponential at 2° C. to 5° C., but at room temperature the rate of growth progressively falls off and ceases altogether after 6 weeks.

3. Transference of food reserves from the endosperm to the embryo takes place only at low temperature, and it is suggested that starvation prevents normal growth at room temperature.

Acknowledgements

This work was carried out in the University of Bristol. I wish to thank Professor M. Skene, of the Botany Department, for suggesting the problem

and for his constant help and advice. I am also indebted to the Agricultural Research Council for a grant which enabled this work to be undertaken.

Literature Cited

Crocker, W., 1948: Growth of Plants, pp. 67–139.

—— and Barton, L. V., 1948: Twenty Years of Seed Research at the Boyce Thompson Institute for Plant Research.

Hant, I. C., 1933: Effects of Various Low Temperatures on Fruit Tree Seeds. Proc. Amer. Soc. Hort. Sci., xxx. 365–7.

Roberts, H. F., 1924: Germination of Seeds exposed to Low Temperature. Nature, cxiv. 393.

Steinbauer, G. P., 1937: Dormancy and Germination of Fraxinus Seeds. Plant Physiol., xii. 813–24.

Stokes, Pearl, 1950: A Study of Germination of *Heracleum sphondylium* L. Ph.D. thesis. University of Bristol, 1950.